AR201-13450A

The Flavor And Fragrance High Production Volume Consortia

The Aromatic Consortium

Test Plan For Benzyl Derivatives

Benzaldehyde	**CAS No. 100-52-7**
***p*-Methoxybenzaldehyde**	**CAS No. 123-11-5**
***m*-Methoxy-*p*-hydroxybenzaldehyde**	**CAS No. 121-33-5**
Benzyl acetate	**CAS No. 140-11-4**
Benzyl benzoate	**CAS No. 120-51-4**
Methyl benzoate	**CAS No. 93-58-3**
Methyl *p*-methylbenzoate	**CAS No. 99-75-2**
Methyl 2-hydroxybenzoate	**CAS No. 119-36-8**
Pentyl 2-hydroxybenzoate	**CAS No. 2050-08-0**
Benzyl 2-hydroxybenzoate	**CAS No. 118-58-1**

FFHPVC Aromatic Consortium Registration Number [redacted]

Submitted to the EPA under the HPV Challenge Program by:
The Flavor and Fragrance High Production Volume Chemical Consortia
1620 I Street, NW, Suite 925
Washington, DC 20006
Phone: 202-331-2325
Fax: 202-463-8998

List of Member Companies

BASF

Eastman Chemical Company

Firmenich, Incorporated

Givaudan Corporation

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International Flavor & Fragrances, Inc.

KoSa

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Noveon, Inc.

Polarome International

Proctor & Gamble

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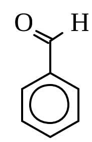
The HPV Challenge Test Plan for Benzyl Derivatives

1 IDENTITY OF SUBSTANCES



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Synonyms: Benzoic aldehyde
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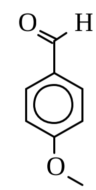

***p*-Methoxybenzylaldehyde**
Synonyms: *p*-Anisaldehyde
$C_8H_8O_2$
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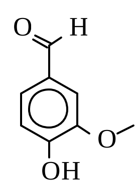

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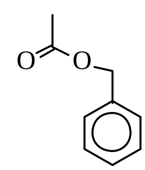

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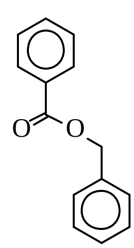
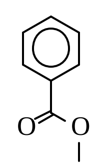


Benzyl benzoate
Synonyms: Benzoic acid, benzyl ester
$C_{14}H_{12}O_2$
CAS No. 120-51-4



Methyl benzoate
Synonyms: Benzoic acid, methyl ester
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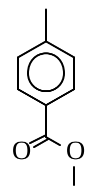
CAS No. 93-58-3



Methyl *p*-methylbenzoate
Synonyms: Methyl p-toluate
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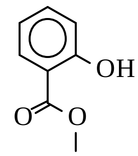

Methyl 2-hydroxybenzoate
Synonyms: Methyl salicylate
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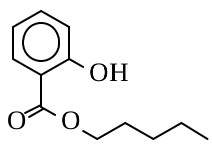

Pentyl 2-hydroxybenzoate
Synonyms: Amyl salicylate
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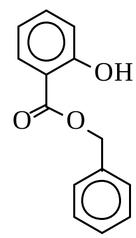


Benzyl 2-hydroxybenzoate
Synonyms: Benzyl salicylate
$C_{14}H_{12}O_3$
CAS No. 118-58-1

2 CATEGORY ANALYSIS

2.1 INTRODUCTION

In October of 1999, members of the U.S. flavor and fragrance industries as well as other manufacturers that produce source materials used in flavors and fragrances formed consortia of companies in order to participate in the Chemical "Right-to-Know" Program. Members of these consortia committed to assuring the human and environmental safety of substances used in flavor and fragrance products. The consortia are organized as the Flavor and Fragrance High Production Volume Consortia (FFHPVC). The Aromatic Consortium, as a member of the FFHPVC serves as an industry consortium to coordinate testing activities for aromatic substances under the Chemical "Right-to-Know" Program. Fourteen (14) companies are current members of the Aromatic Consortium. The Aromatic Consortium and its member companies are committed to assembling and reviewing available test data, developing and providing test plans for each of the sponsored chemicals, and, where needed, conducting additional testing. The test plan, category analysis and robust summaries presented below are the first phase of the Aromatic Consortium's commitment to the Chemical "Right-to-Know" Program.

2.2 BACKGROUND INFORMATION

This chemical category contains members that are some of the most widely used materials in flavors and fragrances and are widely distributed in the food supply. Some members of this category have common names derived from the foods in which they occur. Benzaldehyde being the almost exclusive constituent of bitter almond oil is often called such. *p*-Methoxybenzaldehyde and *m*-methoxy-*p*-hydroxybenzaldehyde are commonly known as *p*-anisaldehyde and vanillin because of their presence in the spices, anise and vannila, respectively. Of the esters in this category methyl 2-hydroxybenzoate or methyl salicylate is widely recognized as oil of wintergreen while the pentyl and benzyl 2-hydroxybenzoate are often called amyl salicylate and benzyl salicylate, respectively. Methyl *p*-methylbenzoate is often called methyl *p*-toluate.

Benzaldehyde can be found in relatively high concentration in many foods including tomatoes, coffee and bread [Stofberg and Grundschober, 1987], but occurs in highest concentration in spices and natural extracts (*i.e*., almond oil and vanilla extract) present on the kitchen shelves of most households [Maarse *et al.*, 1996]. In addition to its presence in vanilla extract, *m*-methoxy-*p*-hydroxybenzaldehyde or vanillin occurs naturally in coffee, berries, and alcoholic beverages [Stofberg and Grundschober, 1987]. Likewise, benzyl and benzoate esters are common naturally occurring components of traditional food. Most notable is methyl 2-hydroxybenzoate or methyl salicylate, the predominant component in oil of wintergreen used in food and over-the-counter health care products (e.g., Ben-Gay and mouth washes). Methyl 2-hydroxybenzoate occurs naturally in fruits, coffee, tea, and alcoholic beverages. Methyl benzoate, benzyl benzoate, benzyl 2-hydroxybenzoate, pentyl 2-hydroxybenzoate can be found in many plant essential oils [Bauer and Garbe, 1985].

In industry wide surveys [Lucas *et al.*, 1999; RIFM, 2000], the volume of use of benzaldehyde, benzyl acetate, *m*-methoxy-*p*-hydroxybenzaldehyde, methyl 2-hydroxybenzoate, and benzyl 2-hydroxybenzoate as flavors and fragrances each exceeds 1,000,000 pounds annually. Other members of the category have an annual volume of use exceeding 100,000 pounds. The annual volume of use of *m*-methoxy-*p*-hydroxybenzaldehyde as an added flavoring substance exceeds 1,000,000 kg [Lucas *et al.,* 1999].

The importance of these substances in food flavors and in fragrances has resulted in numerous scientific reviews and safety evaluations of these substances by national and international regulatory bodies. The U.S. Food and Drug Administration (FDA) recognizes that these substances are generally recognized as safe (GRAS) under conditions of intended use (Code of Federal Regulation, Part 21, Section 172.515) as flavoring substances. The Joint Expert Committee on Food Additives (JECFA) as part of the World Health Organization has regularly reviewed the safety of important members of this category and has assigned acceptable daily intakes (ADIs) for their safe use in food. JECFA has evaluated the benzyl derivatives, including benzoic acid, benzaldehyde, benzyl acetate, and other benzyl and benzoate esters as a group and assigned a group ADI of 5 mg/kg (equivalent to a daily intake of 300 mg/person per day) [JECFA, 1996]. Presumably, if U.S. population consumed the maximum intake daily, the total annual consumption of benzyl derivatives in the U.S. would approach 30,000,000 kg. This would be augmented by the significant quantities of benzyl derivatives consumed as naturally occurring components of food.

Like benzyl derivatives, hydroxy and alkoxybenzene derivatives have been the subject of scientific review and regulatory approval. *m*-Methoxy-*p*-hydroxybenzaldehyde (vanillin), the corresponding ethoxy derivative (ethyl vanillin), and methyl 2-hydroxybenzoate (methyl salicylate) are recognized as safe under intended conditions of use as flavoring substances (CFR, Part 21, Section 172.515). ADIs of 0-10, 0-3, and 0-0.5 mg/kg bw have been assigned to vanillin [JECFA, 1967], ethyl vanillin [JECFA, 1995], and methyl 2-hydroxybenzoate [JECFA, 1967], respectively.

2.3 STRUCTURAL CLASSIFICATION

The chemical category designated "Benzyl Derivatives" includes three benzaldehyde derivatives (benzaldehyde, *p*-methoxy, and *m*-methoxy-*p*-hydroxybenzaldehyde), two benzyl (benzyl acetate and benzyl benzoate) and two benzoate (methyl benzoate and methyl *p*-methylbenzoate) esters, and three 2-hydroxybenzoate esters (methyl, pentyl, and benzyl 2-hydroxybenzoate). The benzaldehyde derivatives are readily oxidized to the corresponding benzoic acid derivatives while the benzyl esters are hydrolyzed to yield benzyl alcohol that is subsequently oxidized to benzoic acid as a stable metabolite or end-product. The benzoate and 2-hydroxybenzoates esters are hydrolyzed to yield benzoic acid and 2-hydroxybenzoic acid derivatives, respectively. The 10 substances are placed in the same category because all contain a benzene ring bonded directly to an oxygenated functional group (aldehyde or ester) that is hydrolyzed and/or oxidized to a benzoic acid derivative. As a stable animal metabolite, benzoic acid derivatives are efficiently excreted primarily in the urine. These reaction pathways have been reported in both aquatic and terrestrial species. The similarity of their toxicologic properties is a reflection their participation in these common metabolic pathways.

2.4 PRODUCTION OF BENZYL DERIVATIVES

Petroleum products provide toluene and benzene as raw materials for synthesis of the various substances in this chemical category. Toluene is oxidized to benzaldehyde and benzoic acid, the latter being a starting material for preparation of the benzoate ester, methyl benzoate. In a similar manner, *p*-xylene is selectively oxidized and esterified to yield methyl *p*-methylbenzoate. Toluene is also a source of benzyl alcohol either directly or through intermediate conversion to benzyl chloride. Benzyl alcohol is esterified to produce the corresponding acetate and benzoate esters. Similarly, *p*-cresol is converted to the corresponding methyl ether and then oxidized to *p*-methoxybenzaldehyde. Phenol is the raw material for preparation of 2-hydroxybenzoic acid (salicylic acid). The acid is subsequently esterified to produce methyl 2-hydroxybenzoate, pentyl 2-hydroxybenzoate, and benzyl 2-hydroxybenzoate. The industrial synthesis of these aromatic chemicals is economically favorable to obtaining these products from natural sources.

2.5 CHEMICAL REACTIVITY AND METABOLISM

2.5.1 Absorption, Distribution, and Excretion

In general, members of this group (benzyl derivatives) are rapidly absorbed through the gastrointestinal tract, metabolized primarily in the liver, and excreted in the urine either unchanged or as conjugates of benzoic acid derivatives [Jones *et al.*, 1956; Davison, 1971; Abdo *et al.*, 1985; Temellini, 1993]. At high doses, conjugation pathways (*e.g.*, glycine) may be saturated; in which case, free benzoic acid is excreted unchanged.

Absorption, distribution and excretion studies have been conducted with 2 members of this group (benzyl acetate and *m*-methoxy-*p*-hydroxybenzaldehyde) and several structural relatives (benzoic acid, benzyl alcohol, 4-hydroxybenzyaldehyde, 4-hydroxybenzoic acid, 2-hydroxybenzoic acid, 2,4-dihydroxybenzyaldehyde, butyl *p*-hydroxybenzoate, 3,4-dimethoxybenzaldehyde, sodium benzoate, and 2-hydoxybenzaldehyde). These substances exhibit remarkably similar patterns of pharmacokinetics and metabolism.

2.5.1.1 Rats and Mice

Groups of male F344 rats and B6C3F1 mice were administered ^{14}C-benzyl acetate orally at levels up to 500 and 1,000 mg/kg bw, respectively, 5 days/week for a period of two weeks [Abdo *et al.*, 1985]. The compound was readily absorbed from the gastrointestinal tract of both species, and approximately 90% and 0.3-1.3% of the total dose was recovered as hippuric acid in the urine and feces, respectively, within 24 hours. No benzyl acetate-derived radioactivity was detected in any tissue (i.e., blood, liver, muscle, adipose, skin, lung, kidney and stomach) analyzed at 24 hours. The clearance pattern was not affected at any dose tested. Such complete clearance indicates that benzyl acetate is readily absorbed and excreted.

^{14}C-Benzyl acetate administered by gavage to groups of male F344 rats at doses of 5, 250, or 500 mg/kg bw as the substance alone, in corn oil, or propylene glycol, resulted in excretion of 70-89% of the dose in the urine within 24 hours [Chidgey and Caldwell, 1986]. Only about 4% of the radioactivity was detected in the feces after 72 hours. Independent of the vehicle, the elimination of benzyl acetate and metabolites, was essentially complete after 3 days. No benzyl acetate was detected in the plasma or urine; however, small amounts of benzyl alcohol were detected in the plasma. At the highest dose, benzoic acid was the major plasma metabolite while at the lowest dose; hippuric acid was the major urinary metabolite. Of the metabolites, the proportion of the benzoic acid glucuronic acid conjugate increased with increasing dose, while low levels (1.0-3.6%) of benzoic acid and benzylmercapturic acid were not affected by dose or vehicle.

To determine the effects of age on disposition of benzyl acetate, 3 to 4-, 9-, and 25-month-old F344 rats and 2-, 13-, and 25-month-old C57BI/6N mice were given a single oral dose of ^{14}C-benzyl acetate at doses of 5 or 500 mg/kg bw (rats) or 10 mg/kg bw (mice) [McMahon *et al.*, 1989]. In rats, approximately 80% of radioactivity was recovered in the urine in the first 24 hours for all age groups. The major urinary metabolite was hippuric acid (percentage excreted was not affected by age) and a minor urinary metabolite was benzylmercapturic acid (percentage excreted was slightly increased in 25-month-old rats). The percentage of radioactivity excreted in the feces was slightly decreased in the 25-month-old group. In mice, hippuric acid was the major urinary metabolite (93-96% of the total dose, with lower percentages excreted in 25-month-old mice than in the younger groups). Fecal excretion was a minor route of elimination and was independent of age. The authors concluded that formation of hippuric acid is not affected by age, but aging does affect the minor routes of metabolism and excretion of benzyl acetate in rats and mice.

F344 rats and B6C3F1 mice were used to study the effect of gavage *versus* dietary administration on the toxicokinetics of benzyl acetate [Yuan *et al*., 1995]. Groups of F344 rats were given a single dose of 500 mg/kg bw of benzyl acetate by gavage in corn oil or were fed diets containing 2,700 ppm (approximately 648 mg/kg bw/d) benzyl acetate for 7 days. Similarly, groups of B6C3F1 mice were given benzyl acetate, 1,000 mg/kg bw by gavage in corn oil or were fed diets containing 10,800 ppm benzyl acetate (approximately 900 mg/kg bw/d) for 7 days. Plasma levels of benzyl alcohol, benzoic acid and hippuric acid were measured at 24-hour intervals. Benzyl acetate was undetectable in the plasma after gavage (after 5 minutes in mice and 10 minutes in rats) or dietary administration. Peak plasma levels of benzoic acid and hippuric acid were reached within 3 hours of gavage administration. Compared to the gavage mode of administration, peak plasma concentrations of benzoic acid were 40-fold less in rats and 300-fold less in mice after dietary administration. Plasma concentrations of hippuric acid were similar regardless of the mode of administration.

Male albino rats were administered 4-hydroxy-3-methoxybenzaldehyde, 100 mg/kg bw in a solution of propylene glycol and water by stomach tube [Strand and Scheline, 1975]. Only trace amounts of benzoic acid derivatives remained in the urine after the first 24 hours and none after 48 hours. Free and conjugated forms of 4-hydroxy-3-methoxybenzoic acid and 4-hydroxy-3-methoxybenzyl alcohol identified in the urine represented 94% of the dose. 4-Hydroxy-3-methoxybenzaldehyde and its primary reduction and oxidation metabolites also were excreted in appreciable amounts in the bile. Bile collected by cannulation for 5 hours after 2 rats were given 100 or 300 mg/kg bw oral doses of 4-hydroxy-3-methoxybenzaldehyde contained glucuronide conjugates of parent aldehyde (6%), corresponding alcohol (8%), and acid (9%).

In Sprague-Dawley albino rats given 100 mg/kg bw of 4-hydroxy-3-methoxybenzaldehyde in 0.9% NaCl by intraperitoneal injection, 60% of the dose was recovered in the 24-hour urine as unconjugated 4-hydroxy-3-methoxybenzoic acid, the sulfate and glucuronic acid conjugates of the acid, conjugates of 4-hydroxy-3-methoxybenzaldehyde, 4-hydroxy-3-methoxybenzyl alcohol, and catechol [Wong and Sourkes, 1966]. The presence of the urinary glycine conjugate of corresponding acid was not reported in this study.

Following administration of 375 mg/kg bw orally to rats or by intraperitoneal injection to mice of ^{14}C-benzoic acid, 88-89% of the radioactivity was recovered in the urine within 24 hours and 91-94% after 72 hours [Nutley, 1990]. Only 1-6% was present in the feces.

Female albino rats injected intraperitoneally with 52.4 mg 2,4-dihydroxybenzaldehyde excreted approximately 6% of the dose in the urine as the corresponding hippurate within 24 hours [Teuchy *et al.,* 1971].

2.5.1.2 Rabbits

Rabbits fed 1,000 mg /kg bw of 4-hydroxy-3-methoxybenzaldehyde by gavage excreted, in the urine, 69% of the dose as free and conjugated 4-hydroxy-3-methoxybenzoic acid, and 14% as conjugated aldehyde [Sammons and Williams, 1941].

4-Hydroxybenzaldehyde was administered as a single dose of 400 mg/kg bw to rabbits [Bray *et al.*, 1952]. Within 24 hours, approximately 75% of the aldehyde dose was excreted in the urine mainly as ether soluble acids, with 27% and 3% as glucuronic acid and sulfate conjugates of 4-hydroxybenzoic acid, respectively. Similarly, 96% of the 4-hydroxybenzaldehyde dose was excreted in the urine as 4-hydroxybenzoic acid and its glycine, glucuronic acid and sulfate conjugates.

Groups of rabbits were administered up to 1,500 mg/kg bw of 4-hydroxybenzoic acid by gavage every 3-7 days [Bray *et al.*, 1947]. Urine was collected continuously with total urinary recovery of the test material in the range of 84-104%. Ether soluble acids comprised 64-75% of the total recovery. Glucuronic acid and sulfate conjugates were also detected in the urine at 10-35% and 4-7%, respectively. Within 24 hours after dosing, the levels for all the metabolites returned to background levels. In a corresponding rabbit study, approximately 94% of a single oral dose of 250 or 500 mg/kg bw of 2-hydroxybenzoic acid was excreted unchanged or as the glucuronic acid and sulfate conjugates [Bray *et al.*, 1948].

Rabbits were administered 200 mg of 3,4-dimethoxybenzaldehyde by stomach tube [Sammons and Williams, 1941]. Within 24 hours, approximately 70% of the dose was recovered in the urine as free corresponding acid (approx. 28%) and its glucuronic acid (approx. 38%) or sulfate (3-7%) conjugate.

2.5.1.3 Dogs

Groups of fasted dogs were orally administered 1,000 mg/kg bw of butyl *p*-hydroxybenzoate, or intravenously injected with 50 mg butyl *p*-hydroxybenzoate/kg bw [Jones *et al.,* 1956]. Blood and urine samples were collected at fixed intervals until the levels returned to background levels within 48 hours. Most of the dose was recovered between 6 and 30 hours after dosing as the *p*-hydroxybenzoic acid conjugate of glucuronic acid at 48% and 40% for the oral and intravenous route, respectively. Although the relatively low rate of recovery seen in both dosing methods was attributed to incomplete hydrolysis of the ester in the body, *in vitro* incubation of the butyl ester with freshly prepared liver homogenate showed complete hydrolysis within 30-60 minutes. Studies conducted with other related benzoate esters, such as methyl and ethyl *p*-hydroxybenzoate, showed significantly higher rates of material recovery suggesting that an increase in the homologous series of alkyl esters may result in the activation of other metabolic and excretion pathways. Overall, the authors concluded that butyl *p*-hydroxybenzoate and other alkyl esters are readily absorbed, metabolized, and excreted by the body.

2.5.1.4 Humans

In humans, 4 full-term and 9 pre-term infants were administered intravenous or intramuscular doses of 0.007-0.222 mmol/kg bw of benzyl alcohol in medication [LeBel *et al.,* 1988]. Pre-term infants had maximum serum concentration levels of benzoic acid approximately 10 times those in full-term infants. Benzoic acid was found at higher percentages in the plasma than hippuric acid regardless of administration route in pre-term infants compared to term infants indicating that glycine conjugation is deficient in pre-term compared to full-term infants.

In humans receiving oral doses of 40, 80, and 160 mg sodium benzoate/kg bw, the clearance of benzoic acid increased disproportionately to dose while the clearance for hippuric acid was proportional to dose [Kubota *et al.*, 1988; Kubota and Ishizaki, 1991]. Peak plasma concentrations of benzoic acid increased with increasing dose, while peak hippuric acid concentrations did not change. The data suggest that the conjugation with glycine to form hippuric acid is a saturable process in humans.

Administration of daily oral doses of 5,330-6,000 mg 2,4-dihydroxybenzoic acid in 1,000 mg doses every 3 hours for 2 to 16 days to patients for the treatment of rheumatic fever resulted in average daily urinary excretion rates of 42.7-75.8 % [Clarke *et al.*, 1958]. Average daily excretion of sulfate conjugate was relatively constant during the study, but average daily excretion of glucuronic acid conjugate increased 4- to 6-fold over the 16-day period.

In humans, an oral dose of 100 mg 4-hydroxy-3-methoxybenzaldehyde dissolved in water revealed an increase in the 4-hydroxy-3-methoxybenzoic acid output in the urine from a background level of 0.3 mg/24 hours to 96 mg/24 hours [Dirscherl and Wirtzfeld, 1964]. The observed increase was approximately 94% of the parent aldehyde dose.

2.5.1.5 Fish

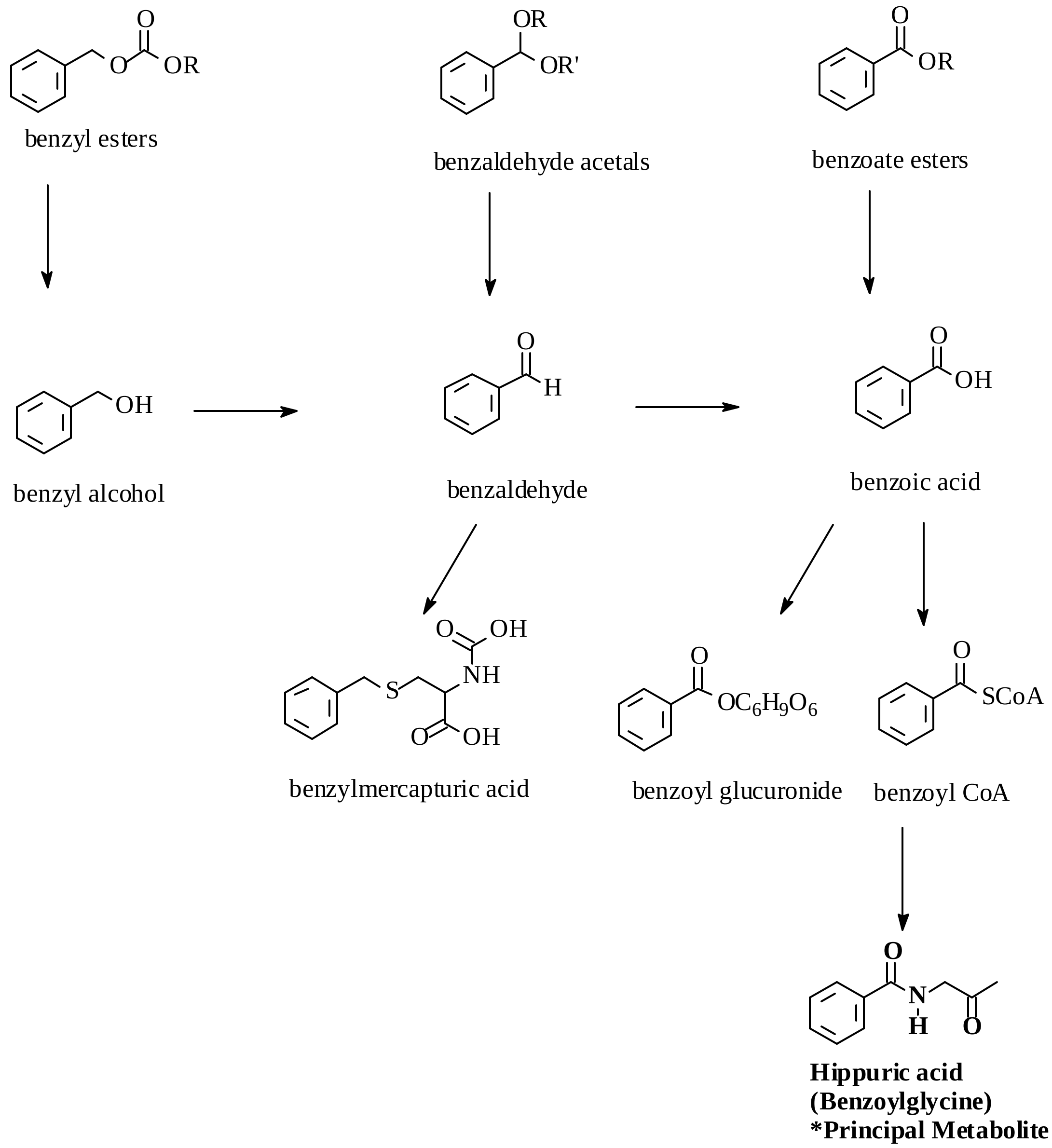
Pharmacokinetic and metabolic studies have been performed on two fish species. In channel catfish, intravascular (iv) or peroral administration of a 10 mg/kg dose of [^{14}C]-benzoic acid was rapidly absorbed and eliminated. After iv dosing, elimination half-life was 5.9 hours, total body clearance was 61 ml/min., and volume of distribution was 369 ml/hr/kg. After oral administration, absorption half-life was only 0.8 hours and bioavailablity was greater than 95%. Greater than 80% of the iv dose was excreted *via* the renal pathway within 24 hours. The major excreted metabolite was the taurine conjugate of benzoic acid [Plakis and James, 1990].

In the southern flounder, greater than 95% of a 15 mg dose of [^{14}C]-benzoic acid given by intramuscular injection was excreted as the taurine conjugate of benzoic acid in the urine. [James and Pritchard, 1987]. The rate of excretion was slow, approximately 10% per day. A subsequent investigation of the transport of benzoic acid, benzoyltaurine, and hippuric acid revealed that, at 100 uM, conjugation of benzoic acid with taurine was slow and there was also saturation of the transport of benzoyltaurine by isolated renal tubules. The amino acid conjugation (*e.g.*, taurine) of benzoic acid has also been studied in rainbow trout (*Salmo gairdneri*) [Burke *et al.*, 1987]. Greater than 99% of the radioactivity derived from a 10 mg/kg dose of [^{14}C]-benzoic acid was given by gelatin capsule was excreted in the urine within 48 hours. Greater than 98% of the excreted radioactivity was accounted for by a single metabolite, benzoyltaurine. Based on these studies, it is concluded that once benzoic acid has been absorbed by fish, is rapidly excreted as the taurine conjugate.

2.5.2 Hydrolysis of Esters

In general, the catalytic activity of carboxylesterases or esterases such as the *beta*-esterases hydrolyzes aromatic esters *in vivo* [Heymann, 1980]. These enzymes are found throughout mammalian tissues, but predominate in hepatocytes [Anders, 1989; Heymann, 1980]. Figure 1 shows the hydrolysis of benzyl and benzoate esters yielding the corresponding alcohols and carboxylic acids, which has been reported in several *in vitro* experiments.

Figure 1. Metabolism of Benzyl Derivatives



In *in vitro* experiments, benzyl acetate was readily hydrolyzed in pig liver homogenate [Heymann, 1980] and reached peak alcohol concentrations after 4 minutes [Yuan *et al*., 1995]. Following the *in vitro* hydrolysis of a series of 4 alkyl benzoates (including methyl benzoate) and 2 aryl benzoates, plasma half-lives ($t_{1/2}$) in 80% human blood plasma decreased from 210 minutes for ethyl benzoate to 24 minutes for butyl benzoate and 19 and 15 minutes for phenyl benzoate and benzyl benzoate, respectively [Nielson and Bundgaard, 1987].

When fed or administered by gavage to rats, no plasma benzyl alcohol was observed; however, high plasma levels of hippuric acid and benzoic acid were detected indicating that benzyl acetate is rapidly hydrolyzed to benzyl alcohol, which is then rapidly oxidized first to benzaldehyde and then to benzoic acid *in vivo* [Yuan *et al*., 1995].

Methyl 2-hydroxybenzoate was orally administered to males rats at a dose equivalent to 500 mg/kg bw of 2-hydroxybenzoic acid [Davison *et al.*, 1961]. Twenty minutes following dosing, plasma levels showed complete hydrolysis of methyl 2-hydroxy benzoate. Similarly, male dogs were given 320 mg/kg bw of the methyl ester in capsules. After one hour, blood samples showed 95% hydrolysis of methyl ester to 2-hydroxybenzoic acid. In humans given 0.42 ml methyl 2-hydroxybenzoate (approximately 500 mg), blood samples showed 79% of the dose hydrolyzed within the first 90 minutes.

Several experiments were conducted [Jones *et al.*, 1956] to study the hydrolysis of *p*-hydroxybenzoic acid esters. Comparisons were made between oral (1,000 mg/kg bw) and intravenous (50 mg/kg bw) administration in dogs. Methyl and ethyl esters were rapidly hydrolyzed by esterases in the liver and kidney. Recovery of the dose of butyl *p*-hydroxybenzoate was 48% and 40% from the oral and intravenous administration, respectively. Liver preparations from dogs injected with 100 mg/kg bw of the methyl, ethyl, or propyl esters showed 100% hydrolysis in 3 minutes; whereas, 100% hydrolysis of the butyl ester occurred after 30-60 minutes.

Carboxylesterase (Type B) activity has been reported in a variety of fish species at different life stages [Leinweber, 1987; Boone *et al*., 1996; Abas and Hayton, 1997;

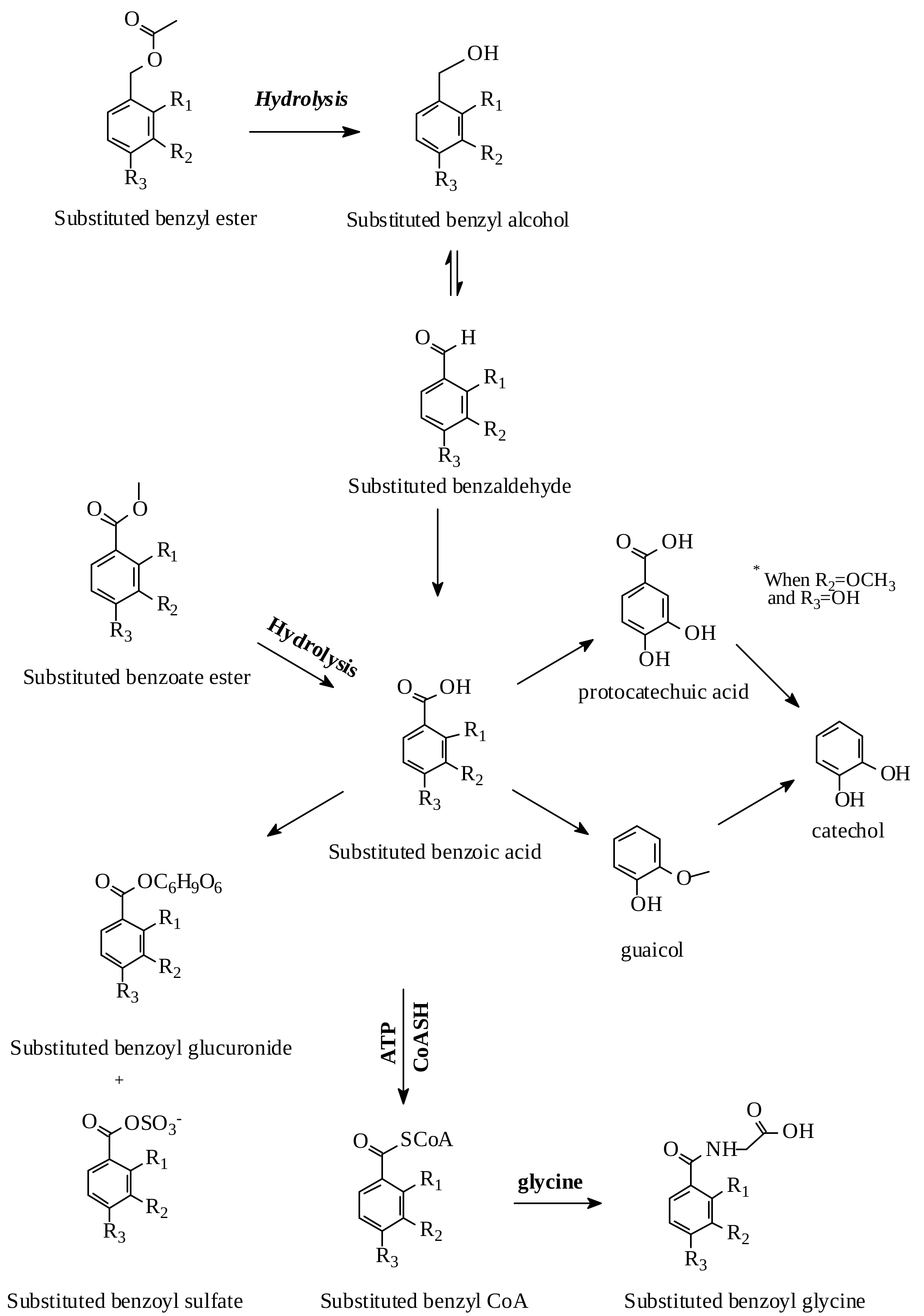
Barron *et al.*, 1999]. Enzyme activity of rainbow trout sera, liver and whole body homogenates were similar to those of rat liver homogenate. A significant increase (300%) in activity occurred between yolk-sac and juvenile stage of rainbow trout development. Carboxylesterase activity was not significantly different for whole body homogenates of the rainbow trout, channel catfish, fathead minnows, and bluegill [Barron *et al.*, 1999]. These data support the conclusion that simple aromatic esters including benzyl acetate, benzyl benzoate, methyl benzoate, methyl *p*-methylbenzoate and methyl, pentyl, and benzyl 2-hydroxybenzoate are readily hydrolyzed in these animals.

In summary, the esters in this chemical category are expected to hydrolyze to the parent alcohols and carboxylic acids *in vivo*. Complete hydrolysis is expected to occur in gastric juice, intestinal fluid, portal blood and liver.

2.5.3 Metabolism of Benzyl Derivatives

In general, benzyl derivatives, including benzyl and benzoate esters, are expected to be hydrolyzed to the corresponding parent alcohol, aldehyde, or acid. The alcohols and aldehydes are oxidized mainly to benzoic acid derivatives that are either excreted unchanged or form sulfate, glycine or glucuronic acid conjugates. Benzyl alcohol oxidizes to benzaldehyde, then benzoic acid. Benzoic acid is then excreted as hippuric acid. Benzyl alcohol may also conjugate with glutathione, but to a lesser extent. Likewise, benzaldehyde may be reduced to benzyl alcohol, and benzoic acid may conjugate with glucuronic acid. As high levels of benzoic acid saturate the glycine (hippurate) conjugation pathway, conjugation with glucuronic acid becomes a more important pathway. At very high levels of exposure, free benzoic acid may sequester significant amounts of acetyl coenzyme A (CoA) leading to disturbances in cellular homeostasis. To some extent, glucuronic acid conjugates may pass into the bile and enter enterohepatic circulation where they are hydrolyzed or undergo reduction reactions with intestinal microflora. Reductive decarboxylation of hydroxy- and alkoxybenzoic acid derivatives has been reported to occur to a minor extent in the gastrointestinal tract. Other minor metabolic detoxication pathways include *O*-demethylation and ring hydroxylation (see Figure 2).

Figure 2. Metabolism of Hydroxy- & Alkoxy-substituted Benzyl Derivatives



Hippuric acid was identified as the major urinary metabolite, with minor amounts of benzyl alcohol, benzoic acid and benzylmercapturic acid, following oral, subcutaneous or intraperitoneal administration of ^{14}C-benzyl acetate to mice (10-1,000 mg/kg bw) and rats (5-500 mg/kg bw) [Abdo *et al*., 1985; Chidgey and Caldwell, 1986; Yuan *et al.*, 1995; Clapp and Young, 1970; McMahon *et al*., 1989].

Groups of male Fischer rats were administered 500 mg /kg bw of ^{14}C-benzyl acetate by gavage, alone or in conjunction with intraperitoneal injections of 200 mg/kg bw of pyrazole (an alcohol dehydrogenase inhibitor), 10 mg/kg bw of pentachlorophenol (a sulfotransferase inhibitor) or both [Chidgey *et al.*, 1986]. Within 24 hours, 92% of the dose of benzyl acetate alone was recovered in the urine, 3% in the feces and less than 1% in the carcasses. The combination treatment of benzyl acetate and inhibitor delayed the excretion of ^{14}C in urine compared to treatment with benzyl acetate alone. The pyrazole only group had significantly higher amounts of benzylmercapturic acid excreted in the first 24 hours. The amount of benzylmercapturic acid was significantly increased in the combination group, but less than the pyrazole only group. These results suggest that benzylmercapturic acid forms *via* the sulfate ester of benzyl alcohol.

Benzyl alcohol was administered in single intraperitoneal injections of 770-1,100 mg/kg bw to CD1 mice [McCloskey *et al.*, 1986]. Within 5 minutes, benzyl alcohol and benzaldehyde were detected in the plasma. Pretreatment with pyrazole resulted in a 200% increase in plasma benzyl alcohol levels, while pretreatment with disulfiram (an aldehyde inhibitor) resulted in a 368% increase in plasma benzaldehyde levels indicating that benzaldehyde and benzyl alcohol are interconvertible in blood plasma.

In the rabbit, single doses of 350 or 750 mg/kg bw of benzaldehyde were excreted in the urine (approximately 83 %) by oxidation to benzoic acid and then excretion predominantly as hippuric acid (approx. 68%) [Laham *et al.*, 1988]. Other urinary metabolites identified were benzoylglucuronic acid (10%), benzoyl glucuronide (3%), free benzoic acid (1.5%), and trace amounts of benzylmercapturic acid.

To a minor extent, benzaldehyde is reduced to benzyl alcohol, which, as the sulfate conjugate, may react with glutathione to form benzylmercapturic acid [Laham and

Potvin, 1987]. Groups of Sprague-Dawley rats (5/group/sex) were administered 400, 750, or 1,000 mg/kg bw/d of benzaldehyde by gavage for 13 consecutive days. After the 2^{nd}, 8^{th}, and 13^{th} dose, 24-hour urinary excretion of benzylmercapturic acid was measured. Although females in the mid- and high-dose groups exhibited a slight decrease in excretion of benzylmercapturic acid after the 8^{th} dose, all groups showed increased urinary levels of the conjugated acid after 13 doses. An increase in dose from 400 to 1,000 mg/kg bw/d resulted in a 7- to 8-fold increase in excreted benzylmercapturic acid.

Administration of 375 mg/kg bw of [^{14}C]-benzoic acid to mice (intraperitoneal) and rats (oral) resulted in excretion of hippuric acid (70.2-84.2%), benzoyl glucuronide (0.7-1.8%), benzoic acid (0.4-12.8%), and 3-hydroxy-3-phenylpropionic acid (0.1-0.2%) [Nutley, 1990].

Hippuric acid was the primary urinary metabolite following oral administration of 1-400 mg/kg bw of [^{14}C]-benzoic acid to various species including primates, pigs, rabbits, rodents, cats, dogs, hedgehogs, bats, birds, and reptiles [Bridges *et al.*, 1970]. The ornithine conjugate of benzoic acid, ornithic acid, was the major urinary metabolite excreted within 24 hours in chickens and reptiles. Benzoyl glucuronide was predominant in the fruit bat. In humans, greater than 99% ^{14}C was excreted as hippuric acid within 24 hours.

Doses of 2,000-5,000 mg sodium benzoate were orally administered to male volunteers [Amsel and Levy, 1969]. At 5,000 mg, a 5,000 mg glycine supplement was administered one hour later and 2,000 mg supplements were given every 2 hours thereafter. Benzoate was excreted mainly as hippuric acid. No free benzoic acid was detected. Minor amounts of benzoyl glucuronide were detected, with more formed at the highest dose. Glycine supplementation increased the rate of hippuric acid excretion, indicating that at high dose levels, glycine is rate limiting for formation of hippuric acid.

p-Methoxybenzaldehyde was incubated for 46 hours with rat cecal preparations [Scheline, 1972]. Analysis revealed the presence of anisic acid and anisyl alcohol indicating that *p*-methoxybenzaldehyde undergoes oxidation and reduction in cecal preparations. *In vivo,* when rabbits were administered 2,000 mg/kg bw of *p*-

methoxybenzaldehyde, within 24 hours, approximately 75% of the dose was excreted as the glucuronic acid conjugate of *p*-methoxybenzoic acid (anisic acid) [Sammons and Williams, 1946].

Similarly, when *p*-methoxybenzyl alcohol was incubated with rat cecal extract for approximately 46 hours, analysis showed the presence of corresponding acid [Scheline, 1972]. *O*-Demethylation was not detected.

In *Actinomyces aureus* A-94, *p*-methoxybenzyl alcohol was oxidized to corresponding acid then demethylated and hydroxylated to yield protocatechuic acid (3,4-dihydroxybenzoic acid) [Tsai *et al.*, 1965]. Protocatechuic acid was converted to succinic acid via *beta*-carboxymuconic acid and *beta*-oxoadipic acid and then entered the tricarboxylic cycle.

m-Methoxy-*p*-hydroxybenzaldehyde administered to male rats as single oral dose of 100 or 300 mg/kg bw was excreted in the urine mainly as the parent aldehyde, *m*-methoxy-*p*-hydroxybenzoic acid, and *m*-methoxy-*p*-hydroxybenzyl alcohol within the first 24 hours [Strand and Scheline, 1975]. Minor amounts of *O*-demethylated, decarboxylated and further reduced metabolites were also identified including protocatechuic acid (3,4-dihydroxybenzoic acid, product of *O*-demethylation), guaiacol (*o*-methoxyphenol, product of decarboxylation), the glycine conjugate of *m*-methoxy-*p*-hydroxybenzoic acid, catechol (*o*-hydroxyphenol), 4-methylguaiacol (product of alcohol functional group reduction), and 4-methylcatechol (product of reduction and ring hydroxylation). Only traces of *m*-methoxy-*p*-hydroxybenzoic acid derivatives were detected in the urine collected between the 24 to 48-hour period, and no metabolites were detected in urine collected after 48 hours.

In rabbits administered 100 mg/kg bw of *m*-methoxy-*p*-hydroxybenzaldehyde by intraperitoneal injection, approximately 69% was oxidized to *m*-methoxy-*p*-hydroxybenzoic acid and 10% was reduced to the corresponding alcohol and excreted within 24 hours in the urine [Sammons and Williams, 1941]. Over 10% of the dose was excreted as the glucuronic acid conjugate of the unchanged parent aldehyde.

m-methoxy-*p*-hydroxybenzoic acid, and the toluene derivatives, 4-methylguaiacol and 4-methylcatechol, were identified following incubation of *m*-methoxy-*p*-hydroxybenzyl alcohol with rat cecal extract [Scheline, 1972]. Microflora-mediated metabolic transformations included reduction, dehydroxylation, *O*-demethylation, and decarboxylation leading to a variety of benzyl alcohol, benzoic acid, and toluene derivatives.

In vivo, administration of 100 or 300 mg/kg bw of *m*-methoxy-*p*-hydroxybenzyl alcohol by gavage to male rats also resulted in the presence of unconjugated and glycine conjugated *m*-methoxy-*p*-hydroxybenzoic acid along with trace amounts of parent alcohol in 24-hour urine [Strand and Scheline, 1975]. Also identified in smaller quantities were conjugated fractions of the corresponding aldehyde, guaiacol, catechol, 4-methylguaiacol and 4-methylcatechol. The presence of catechol and 4-methylcatechol indicate that decarboxylation and complete reduction of the alcohol function, respectively, occur *in vivo*, probably in the gut.

In rabbits, gavage administration of 2,000 mg/kg bw of 3,4-dimethyoxybenzaldehyde (veratraldehyde) showed approximately 70% of the dose excreted in 24-hour urine mainly as the corresponding acid, veratric acid (28%) and its glucuronic acid conjugate (38%) [Sammons and Williams, 1946]. To a lesser extent, veratric acid was decarboxylated and *O*-demethylated to yield catechol. Presumably, veratric acid enters enterohepatic circulation where intestinal microflora decarboxylate the acid to yield catechol (*o*-hydroxyphenol). The observation that catechol, is formed when veratraldehyde is incubated with rat cecal extract is evidence for this decarboxylation pathway in intestinal microflora [Scheline, 1972].

Rats intraperitoneally injected with 52 mg 2,4-dihydroxybenzoic acid, excreted less than 6% of the dose as the corresponding hippurate [Teuchy *et al.*, 1971].

In humans, daily oral doses of up to 6000 mg 2,4-dihydroxybenzoic acid for up to 16 days were primarily excreted in the urine as its glucuronic acid and sulfate conjugates [Clarke *et al*., 1958].

In conclusion, the benzyl, benzoate, and 2-hydroxybenzoate esters in this category are hydrolyzed to the corresponding alcohols and carboxylic acids. The benzyl alcohol and benzaldehyde derivatives are oxidized to the corresponding benzoic acid derivatives that are subsequently excreted unchanged or as glycine or glucuronic acid conjugates. If methoxy or phenolic functional groups are present on the benzene ring, additional minor metabolic options become available. *O*-demethylation yields the corresponding phenol that is subsequently excreted as the glucuronic acid or sulfate conjugates. At high dose levels, gut microflora may act to produce minor amounts of reduction metabolites.

3 TEST PLAN

3.1 PHYSICAL AND CHEMICAL PROPERTIES

3.1.1 Melting Point

Literature values from reliable sources are available for 8 of the 10 substances in this group. For the three aldehydes in this category, the progression of melting points from -26 ^{o}C for benzaldehyde to 80-81 ^{o}C *m*-methoxy-*p*-hydroxybenzaldehyde are expected based on increasing molecular weight and polarity [Merck, 1996; CRC, 2000]. Measured melting points for the four benzyl and benzoate esters (-12.4 to 33.2 ^{o}C) also are also related to the molecular weights of the esters [Merck, 1996; CRC, 2000]. The measured melting point for methyl 2-hydroxybenzoate (-8 ^{o}C) [Merck, 1996] and the calculated melting points [US EPA (EPI) MPBPWIN, 2000] for benzyl 2-hydroxybenzoate (130.5 ^{o}C) and pentyl 2-hydroxybenzoate (82.5 ^{o}C) are consistent with the chemical structure and the measured value and are considered acceptable.

3.1.2 Boiling Point

Literature values from reliable sources are available for all 10 substances in this group. While none of the reported boiling points was obtained according to currently recognized guidelines, the consistency of the values reported by standard reference sources [Arctander, 1969; Merck, 1996; FMA; CRC, 2000] mutually confirm their reliability. The three aldehydes exhibit boiling points proportional to their molecular weight and polarity (benzaldehyde, 179 ^{o}C; *p*-methoxybenzaldehyde, 248 ^{o}C; *m*-methoxy-*p*-hydroxybenzaldehyde, 285 ^{o}C). Boiling points for the four benzyl and benzoate esters reflect the increasing molecular weights of these substances. Likewise, the reported boiling points of 220-224 ^{o}C, 277-278 ^{o}C, and 320 ^{o}C for the methyl, pentyl, and benzyl esters of 2-hydroxybenzoic acid respectively, reflect their increasing molecular weights [Merck, 1996; CRC, 2000].

3.1.3 Vapor Pressure

Measured values are available for 7 of the 10 substances in this group. The close correlation of measured and calculated values give a high degree of confidence in the algorithm for this group and for the calculated values for the remaining 3 substances.

The measured vapor pressures of 0.169 kPa for benzaldehyde [Ambrose *et al.*, 1975], 0.0044 kPa for *p*-methoxybenzaldehyde [Ohe, 1989], and 0.00002 kPa for *m*-methoxy-*p*-hydroxybenzaldehyde [Yaws, 1994], are in good agreement with calculated values of 0.134, 0.0041, and 0.00006 kPa, respectively [US EPA (EPI) MPBPWIN, 2000]. The measured vapor pressure of 0.024 kPa for benzyl acetate, 0.00007 kPa for benzyl benzoate, and 0.0507 for methyl benzoate [Daubert and Danner, 1986] are within ± 0.001 kPa of calculated values [US EPA (EPI) MPBPWIN, 2000]. Based on these data, the calculated vapor pressure of 0.014 kPa for methyl *p*-methylbenzoate is anticipated to be reliable [US EPA (EPI) MPBPWIN, 2000]. The measured vapor pressure of methyl 2-hydroxybenzoate and benzyl 2-hydroxybenzoate are reported to be 0.005 kPa [Daubert and Danner, 1989] and 0.00013 kPa [FMA], respectively. The values are in good agreement with calculated values of 0.0072 and 0.0000024 kPa, respectively [US EPA (EPI) MPBPWIN, 2000], and confirm the calculated vapor pressure of 0.0009 kPa for pentyl 2-hydroxybenzoate [US EPA (EPI) MPBPWIN, 2000].

3.1.4 Octanol/Water Partition Coefficients

Measured values are available for 8 of the 10 substances in this category. The close correlation of these measured values with those calculated by the method of Meylan (1993-1995) give a high degree of confidence in the algorithm for this group and for the calculated values for the remaining 2 substances.

The measured log Kow values [Hansch *et al.*, 1995] of 1.48, 1.76, and 1.21 for benzaldehyde, *p*-methoxybenzaldehyde, *m*-methoxy-*p*-hydroxybenzaldehyde, respectively, are in close agreement with calculated values of 1.71, 1.79, and 1.05 [US EPA (EPI) KOWWIN, 2000]. The decreasing trend of values (1.76 to 1.48 to 1.21) among the three benzaldehyde derivatives reflects addition of polar and nonpolar functional groups to the benzene ring. Likewise measured log Kow values of 1.96 for benzyl acetate [Sangster, 1989], 3.97 for benzyl benzoate [Sangster, 1989], 2.20 for methyl benzoate [Sangster, 1989], and 2.70 for methyl *p*-methylbenzoate [Sotomatsu *et al.*, 1993] are within ± 0.4 log Kow units of calculated values [US EPA (EPI) KOWWIN, 2000]. The log Kow value of 2.55 for methyl 2-hydroxybenzoate [Sangster, 1994] is closely correlated with the calculated value of 2.60 [US EPA (EPI) KOWWIN, 2000]. The calculated log Kow values for the benzyl (4.31) and amyl (4.57) [US EPA (EPI) KOWWIN, 2000] 2-hydroxybenzoate are judged reliable based on the value for the methyl ester and given the influence of pentyl and benzyl moieties on the solubility in octanol.

3.1.5 Water Solubility

Measured values are available for 6 [Yalkowski and Dannenfelser, 1992; Riddick *et al.*, 1986; Chem Inspect Test Inst, 1992] of the 10 substances in this group. The good agreement of these measured values with those calculated by the method of Meylan (1993-1995) give a high degree in confidence in the algorithm for this group and for the calculated values for the remaining 4 benzyl derivatives.

3.1.6 New Testing Required

None required.

3.2 ENVIRONMENTAL FATE AND PATHWAYS

3.2.1 Photodegradation

The calculated half lives for hydroxyl radical reactions (AOPWin) range from 4.7 to 64.5 hours. The calculated photodegradation half-lives for three benzaldehyde derivatives in this chemical category are in the narrow range from 4.7 hours for *m*-methoxy-*p*-hydroxybenzaldehyde to 7.2 hours for the less substituted derivative benzaldehyde [US EPA (EPI) AOPWIN, 2000]. The relative half-lives reflect the increased reactivity of a phenolic OH group. The half-lives for the aldehydes are shorter than those for the corresponding benzyl and benzoate esters in this category (12.7 for methyl benzoate to 64.5 hours for methyl *p*-methylbenzoate) [US EPA (EPI) AOPWIN, 2000]. Generally, the carboxylate function of the ester is more stable to reaction with hydroxyl radicals than is the aldehyde function. The presence of an aromatic phenol function capable of undergoing ready hydrogen abstraction of the phenolic hydroxyl group decreases predicted photodegradation half-lives. Therefore, the methyl, pentyl and benzyl esters of 2-hydroxybenzoic acid have calculated half-lives of 11.6, 7.6, and 7.4 hours, respectively.

3.2.2 Stability in Water

The three benzaldehydes in this group cannot hydrolyze. However, they are likely to be slowly oxidized to their corresponding acids. The calculated hydrolysis half-lives for the remaining 7 esters range from 20 days at pH 8 and 198 days at pH 7 for benzyl acetate to 1.1 years at pH 8 and 10.8 years at pH 7 for methyl *p*-methylbenzoate. Benzyl acetate, like several other benzyl esters, was shown to be 50% hydrolyzed in 2 hours at pH 7.5 in the presence of pancreatin in an *in vitro* simulation experiment (see Section 2.5.2). These esters are all expected to be readily hydrolyzed *in vivo.*

3.2.3 Biodegradation

Measured biodegradability data are available for 8 of the 10 substances in this category [benzaldehyde, *p*-methoxybenzaldehyde, methyl benzoate, benzyl benzoate, benzyl acetate, methyl 2-hydroxybenzoate, benzyl 2-hydroxybenzoate, and pentyl 2-hydroxybenzoate]. All are readily and ultimately biodegradable using a standard OECD 301B test or 301F protocol [Quest, 1994; Muller and Bruns, 1995; Quest, 1995a, 1995b; Corby, 1995; Birch and Fletcher, 1991, 1994; Muller and Caspers, 1992]. In the case of benzyl 2-hydroxybenzoate and pentyl 2-hydroxybenzoate, they were considered readily biodegradable when tested using the Manometric Respirometry Test (OECD 301B or 301F) [Muller and Caspers, 1992; Muller and Bruns, 1995]; but the benzyl ester was not readily biodegradable when tested using the CO_2 Evolution Test [Muller and Bruns, 1993]. In the CO_2 Evolution Test, pentyl 2-hydroxybenzoate was readily biodegradable (62% in 28 days) [Latham *et al.*, 1999]. The parent acid benzoic acid, its sodium salt, and 2-hydroxybenzoic acid were all readily biodegradable in a COD (chemical oxygen demand) test [Birch and Fletcher, 1991; Pitter, 1976]. Since hydrolysis of the esters and ready oxidation of the aldehydes yields corresponding acid derivatives, the data on benzoic acid, its sodium salt, and 2-hydroxybenzoic acid validate the observations that the aldehydes and esters in this chemical category are readily biodegradable. All materials in this group were also predicted to be readily degradable by BIOWIN model calculations [US EPA Estimation Program Interface (EPI) Suite (2000) BIOWIN v4.00, EPA and Syracuse Research Corporation.].

3.2.4 Fugacity

Transport and distribution in the environment were modeled using Level 1 Fugacity-based Environmental Equilibrium Partitioning Model Version 2.11 [Trent University, 1999]. The principal input parameters into the model are molecular weight, melting point, vapor pressure, water solubility, and log Kow. When measured values were available, they were used, but when they were not available, calculated data from the EPIWIN series of programs were used [US EPA Estimation Program Interface (EPI) Suite (2000) ECOSAR].

The model predicts that the three aldehydes are distributed mainly to the water (greater than 92%) with the more polar *m*-methoxy-*p*-hydroxybenzaldehyde having highest distribution to water. Consistent with the low water solubility and high log Kow data the highest percentage aldehyde distributed to soil was for *p*-methoxybenzaldehyde (4.72%). Based on this physiochemical model, the ratio for distribution of the three aldehydes between water (greater than 92%) and fish (0.00008 to 0.00027%) is greater than four orders of magnitude suggesting low bioaccumulation in fish.

In comparison, the four benzyl and benzoate esters exhibit higher partition coefficients and decreased water solubilities than do the three benzaldehyde derivatives. Therefore, it is not unexpected that the model predicts proportionately lower distribution to water and higher air and soil distribution for the group of esters. The distribution to water is in the range from 10.5% for the more lipophilic benzyl benzoate to 76% for benzyl acetate. Distribution to air is in the range from 0.39% for benzyl benzoate to 37% for methyl benzoate reflecting the relative vapor pressures of these substances. Distribution to soil is in the range from 87% for benzyl benzoate to 6.5% for methyl benzoate. The ratio for distribution of the four esters between water/air/soil and fish (0.00037 to 0.0049%) is at least three orders of magnitude, again suggesting low bioaccumulation in fish.

The three 2-hydroxybenzoate esters having lower vapor pressures than the benzyl and benzoate esters, distribute mainly to the soil (20.6 to 93.6%) and water (3.4 to 65.7%). The high soil distribution (92.7 and 93.4% for benzyl and amyl 2-hydroxybenzoate, respectively) may be accounted for by the presence of a phenolic function that readily complexes soil metals (*e.g.* iron). The ratio for distribution of the three esters between soil (20.1-93.6%) and fish (0.0012 to 0.0051) is at least three orders of magnitude.

The significance of these calculations must be evaluated in the context that the substances in this chemical category are readily degraded to the parent benzoic acid derivatives. Benzoic acids and hydroxybenzoic acids are ubiquitous in the environment being present in plants and as the metabolites of amino acids in animals. The model does not account for the influence of background biogenic production on partitioning in the environment nor does it take into account the recognized reactivity (*i.e.*, oxidation) of aromatic aldehydes or hydrolytic potential of the aromatic esters discussed here. Therefore, the relevance of fugacity calculations for these substances must be evaluated in the context of these factors.

3.2.5 New Testing Required

None required.

3.3 ECOTOXICITY

3.3.1 Acute Toxicity to Fish

Experimental data available on 7 of the 10 members of this category and the calculated values for all members indicate a low to moderate toxicity for the group of benzyl derivatives. Calculated model values are consistent with experimental data. The NOEC for benzaldehyde to 1-day and 4-day larvae was reported to be less than 0.9 mg/L and the NOEC for survival of 1-day and 4-day larvae was 3.6 mg/L (first test) while the NOEC for survival in second test was 0.22 mg/L for 1-day larvae and 1.8 mg/L for 4-day and 7-day larvae [Pickering, *et al.*, 1996]. A 14-day LC50 for benzaldehyde in guppies has been reported to be 1.57 µM/L (0.17 mg/L) [Deneer *et al.*, 1988]. Other benzaldehyde derivatives exhibit lower acute toxicity compared to the parent substance. The 96-hour LC50 for *m*-methoxy-*p*-hydroxybenzaldehyde has been reported to be 88 to 116 mg/L depending on the water used [Mattson *et al.*, 1976]. The 96-hour LC50 model values for the benzaldehyde derivatives, benzaldehyde, p-methoxylbenzaldehyde, and m-methoxy-p-hydroxybenzaldehyde are in the range from 13-23.5 mg/L [US EPA Estimation Program Interface (EPI) Suite (2000) ECOSAR].

The 96-hour LC50 values for benzyl esters are in the range from 1-5 mg/L and the 96-hour LC50 range for benzoate esters is greater than 10 mg/L. The 96-hour LC50 for benzyl acetate in Japanese Medaka has been reported to be 4.0 mg/L [Holcombe *et al.*, 1995]. In a semi-static 24-hour renewal test with benzyl acetate, the LC50 to Zebra fish has been reported to be 15.8 mg/L based on nominal concentrations and 7.9 mg/L based on concentrations measured at end of exposure [Kanne, 1994]. In a semi-static test performed using the same protocol, the LC50 of benzyl benzoate in Zebra fish was reported to be 3.9 mg/L based on nominal concentrations and 1.3 mg/L based on concentrations measured at the end of exposure [Kanne, 1993b]. The chronic NOEC of benzyl acetate for Japanese Medaka was also reported as 1.33 mg/L and the NOEC for growth of 7-day larvae was 1.8 mg/L [Holcombe *et al.*, 1995]. For the methyl ester of benzoic acid, the 96-hour LC50 for methyl benzoate in Bluegill sunfish was 28.3 mg/L with a NOEC or 10 mg/L based on mortality and loss of equilibrium [Cunningham, 1997a]. Model LC50 values are approximately 2.5 mg/L for benzyl acetate and benzyl benzoate and order of magnitude (18-23 mg/L) greater for methyl benzoate and methyl *p*-methylbenzoate [US EPA Estimation Program Interface (EPI) Suite (2000) ECOSAR].

Esters of 2-hydroxybenzoic acid (i.e. salicylates) exhibit 96-hour LC50 values lower than those for the corresponding benzoate esters. In semi-static tests, in which the LC50 values were based on mean final concentrations measured after 24 hours of exposure, the 24-hour LC50 of pentyl 2-hydroxybenzoate was reported to be 1.34 mg/L [Bruns, 1993] and the 24-hour LC50 values of benzyl 2-hydroxybenzoate was reported to be 0.55 mg/L [Kanne, 1993a]. Based on nominal concentrations, the 24-hour LC50 values for the pentyl and benzyl esters were 4.7 and 1.7 mg/L, respectively. These LC50 ranges are consistent with model LC50 values of 0.9 mg/L for pentyl 2-hydroxybenzoate and 1.3 mg/L for benzyl 2-hydroxybenzoate [US EPA Estimation Program Interface (EPI) Suite (2000) ECOSAR].

In summary, it appears that benzyl esters and salicylate esters exhibit higher acute toxicity to fish than do benzoate esters or benzaldehyde derivatives. Measured values for benzyl and salicylate esters are in the 1-5 mg/L range with salicylates being slightly more toxic. Benzoate ester and benzaldehyde derivatives exhibit 96-hour LC50 values above 10 mg/L.

3.3.2 Acute Toxicity to Aquatic Invertebrates

Acute toxicity for aquatic invertebrates parallels that for fish in the benzyl chemical category. The 24-hour LC50 for benzaldehyde to *Daphnia magna* has been reported as 50 mg/L [Bringmann and Kuehn, 1977]. The calculated 48-hour LC50 model values for benzaldehyde, *p*-methoxybenzaldehyde, and *m*-methoxy-*p*-hydroxybenzaldehyde are 12, 10, and 17 mg/L, respectively [US EPA Estimation Program Interface (EPI) Suite (2000) ECOSAR].

Benzoate esters exhibit low experimental and calculated acute toxicity to invertebrates. The 48-hour EC50 for methyl benzoate has been reported to be 32.1 mg/L in *Daphnia magna* [Cunningham, 1997b]. The 48-hour LC50 model values for methyl benzoate and methyl *p*-methylbenzoate are calculated to be 84 and 28 mg/L, respectively [US EPA Estimation Program Interface (EPI) Suite (2000) ECOSAR]. Calculated 48-hour LC50 model values for benzyl acetate and benzyl benzoate are 130 and 2.7 mg/L, respectively [US EPA Estimation Program Interface (EPI) Suite (2000) ECOSAR].

The 48-hour EC50 value of pentyl 2-hydroxybenzoate is reported to be 2.8 mg/L for *Daphnia magna* [Haarmann and Reimer, 1999]. Calculated model values for salicylate esters reflect the presence of an ester and a phenol moiety in these substances. Methyl, pentyl, and benzyl esters have calculated 48-hour LC50 values of 38.2, 0.9, and 1.4 mg/L, respectively, as esters and 4.9, 1.1, and 1.4 mg/L when considered as phenols [US EPA Estimation Program Interface (EPI) Suite (2000) ECOSAR].

3.3.3 Acute Toxicity to Aquatic Plants

In the only study of acute effects on aquatic plants, benzaldehyde and *m*-methoxy-*p*-hydroxybenzaldehyde stimulated the respiration of cells from *Chlorella vulgaris* in a dose dependent manner. Oxygen uptake was maximal at lower pH (pH 5.6 versus 7.2) and higher concentrations (0.001 M) [Dedonder and Van Sumere, 1971]. ECOSAR model calculations parallel values for other ecotoxicity endpoints. The 96-hour EC50 model values for the benzaldehyde, *p*-methoxylbenzaldehyde, and *m*-methoxy-*p*-hydroxybenzaldehyde are in the range from 110 to 378 mg/L. The four benzyl and benzoate esters exhibit 96-hour calculated EC50 values from 0.24 (benzyl benzoate) to 1.9 mg/L (benzyl acetate). The methyl, pentyl, and benzyl esters of 2-hydroxybenzoic acid (salicylates) have 96-hour EC50 values of 0.95, 0.13, and 0.17 mg/L, respectively, when considered as esters and 24.6, 0.65 and 1.20 mg/L, respectively, when considered as phenol derivatives [US EPA Estimation Program Interface (EPI) Suite (2000) ECOSAR].

3.3.4 New Testing Required

There is sufficient fish acute toxicity data for benzyl, benzoate and salicylate esters that are consistent with model values.

Acute aquatic invertebrate toxicity data are available for benzaldehyde, methyl benzoate and pentyl salicylate. Additional data recommended to validate the model include tests on *p*-methoxybenzaldehyde or *m*-methoxy-*p*-hydroxybenzaldehyde, and benzyl benzoate.

Because of the general lack of aquatic plant data for this group of substances and what appears to be some inconsistency in the algorithms used in the SAR software to calculate aquatic toxicity, acute plant toxicity studies will be conducted on four additional representative members of the group, benzaldehyde, *p*-methoxybenzaldehyde, benzyl benzoate, and pentyl 2-hydroxybenzoate.

3.4 HUMAN HEALTH DATA

3.4.1 Acute Toxicity

Oral LD_{50} values are available for all 10 benzyl compounds. In rats, the oral LD50 values ranged from 887 to greater than 5,000 mg/kg bw demonstrating the low toxicity of these compounds [Graham and Kuizenga, 1945; Draize *et al.*, 1948; Jenner *et al.*, 1964; Sporn *et al.*, 1967; Smyth *et al.*, 1954; Fogelman *et al.*, 1970; Kravets-Bekker and Ivanova, 1970; Moreno, 1977b; 1982a; Meisel, 1982; Zühlke, 1982; NTP, 1986]. Similar oral LD50 values have also been reported in mice for benzyl acetate, benzyl benzoate, *m*-methoxy-*p*-hydroxybenzaldehyde, methyl benzoate, and methyl 2-hydroxybenzoate and in guinea pigs for benzyl benzoate, benzaldehyde, *p*-methoxybenzaldehyde, m-methoxy-p-hydroxybenzaldehyde, methyl benzoate, and methyl 2-hydroxybenzoate [Draize *et al*., 1948; Jenner *et al.*, 1964; Kravets-Bekker and Ivanova, 1970; Ohsumi *et al*., 1984; NTP, 1986; Inouye *et al*., 1988]. The lowest LD50 values were reported with m-methoxy-p-hydroxybenzaldehyde, methyl 2-hydroxybenzoate, and benzaldehyde. Dogs appear to tolerate benzyl benzoate far better than cats, rats or rabbits as was demonstrated by Graham and Kuizenga (1945) when dogs tolerated over 10 times the dose administered to the other species.

Dermal LD50 values in rabbits exceeded 5,000 mg/kg bw for methyl-*p*-methylbenzoate, *p*-methoxybenzaldehyde, and pentyl 2-hydroxybenzoate [Moreno, 1973b, 1977a, 1982b]. The dermal LD50 value for benzaldehyde was greater than 1.25 mg/kg bw [Moreno, 1973a] and for methyl benzoate exceeded 2,000 mg/kg bw [Merriman, 1995].

Oral LD50 studies are available for all of the compounds in this group, although most of the data were obtained prior to GLP and OECD guidelines. However, because of the close structural relationship of the substances and the similarity of dermal and oral LD50 values [Graham and Kuizenga, 1945], the studies are considered adequate to support this category.

3.4.2 Genetic Toxicity

Overall, *in vitro* and *in vivo* genotoxicity studies have been conducted with substances representing the structural characteristics of the benzyl category. The results of these studies were predominantly negative, as described below. Limited positive and/or equivocal findings have been reported for 3 aldehydes and benzyl acetate, but, in most cases, other studies of the same endpoint with same test substance show no activity. Most importantly, *in vivo* studies on benzaldehyde derivatives and closely related benzyl esters have all yielded negative results. These negative *in vivo* genotoxicity assays are supported by the lack of tumorigenicity in chronic animal studies with representatives of this group. Therefore, the database on genetic toxicity of benzyl derivatives is adequate to support this chemical category.

3.4.2.1 In Vitro

In vitro genetic toxicity data are available on 9 of the substances (benzaldehyde, benzyl acetate, benzyl benzoate, *p*-methoxybenzaldehyde, *m*-methoxy-*p*-hydroxybenzaldehyde, methyl 2-hydroxybenzoate, benzyl 2-hydroxybenzoate, methyl *p*-methyl benzoate and methyl benzoate) in this group and on 5 structural relatives that are either metabolic precursors or principal metabolites the substances in this category {benzyl alcohol, benzoic acid, sodium 2-hydroxybenzoate (sodium salicylate), 2-hydroxybenzoic acid (salicylic acid), and sodium benzoate}. The vast majority of standardized *in vitro* genotoxicity assays (Ames (AMS), mouse lymphoma (MLA), sister chromatid exchange (SCE), chromosomal aberration (ABS), and unscheduled DNA synthesis (UDS)) show no evidence of genotoxicity. Equivocal results have been reported mainly for aromatic aldehydes in the MLA and ABS assays.

The above substances were non-mutagenic in all standard plate incorporation and/or pre-incubation Ames assays using *Salmonella typhimurium* strains TA92, TA94, TA97, TA98, TA100, TA102, TA104, TA1535, TA1537, and TA1538, when tested at concentrations ranging up to the level of cytotoxicity or at ICH/OECD-recommended maximum test concentrations, both in the absence and presence of metabolic activation (S9 fraction) [McCann *et al.,* 1975; Milvy and Garro, 1976; Sasaki and Endo, 1978; Florin *et al*., 1980; Kawachi *et al*., 1980a, 1980b; Rapson *et al.*, 1980; Kasamaki *et al.*, 1982; Wiessler *et al.*, 1983; Ishidate *et al.,* 1984; Nagabhushan and Bhide, 1985 Mortelmans *et al*., 1986; Rogan *et al.*, 1986; Fujita and Sasaki, 1987; Schunk *et al.*, 1988; Zeiger *et al.*, 1987, 1988, 1992; Heck *et al.*, 1989; NTP, 1989; 1990; Dillon *et al.*, 1992, 1998; Kuboyama and Fujii, 1992]. Additional Ames tests on metabolites isolated from the urine of rats administered benzaldehyde by oral gavage also were negative in *Salmonella typhimurium* strains TA98 and TA100, both with and without metabolic activation [Rockwell and Raw, 1979]. Mutation or DNA repair assays using *Escherichia coli* strains WP2 *uvrA* or Sd-4-73 with benzyl alcohol, benzyl acetate, and methyl benzoate [Szybalski, 1958; Yoo, 1986], and *Saccharomyces cerevisiae* strain D61.M with benzyl acetate [Zimmerman *et al.,* 1989] also showed no evidence of genotoxicity.

Negative results were obtained with methyl 2-hydroxybenzoate, p-methoxybenzaldehyde, *m*-methoxy-*p*-hydroxybenzaldehyde, benzaldehyde, and sodium 2-hydroxybenzoate in the Rec DNA repair assay using *Bacillus subtilis* strains H17 and M45, whereas equivocal results were obtained with benzyl acetate and benzyl alcohol, and weakly positive results were obtained with benzoic acid, salicylic acid, sodium benzoate, and metabolically activated benzaldehyde [Oda *et al*., 1978; Kawachi *et al*., 1980a, 1980b; Yoo, 1986; Matsui *et al*., 1989; Kuboyama and Fujii, 1992]. Positive and negative results for Rec assays using the same substance were apparently due to laboratory-specific factors. In one study [Yoo, 1986] only positive findings were reported while in another study [Oda,1978] only negative results were reported for the same compounds.

In vitro assays in isolated mammalian cells produced both negative and positive results. In addition to benzyl acetate, benzaldehyde exhibited evidence of mutagenicity in the forward mutation assay with L5178Y mouse lymphoma cells (MLA), both with and without metabolic activation and *p*-methoxybenzaldehyde induced mutation frequency without metabolic activation [Caspary *et al*., 1988; Garberg *et al*., 1988; Wangenheim and Bolcsfoldi, 1988; McGregor *et al.,* 1991; Heck *et al.,* 1989]. *m*-Methoxy-p-hydroxybenzaldehyde did not induce mutation frequency with or without metabolic activation in the MLA [Heck *et al.*, 1989] and benzyl alcohol only elevated mutation frequency at cytotoxic levels in the absence of metabolic activation [NTP, 1989].

In cytogenetic tests, equivocal results were reported for the benzyl and 2-hydroxybenzoate derivatives in the ABS assay. In the ABS assay, performed in human lymphocytes, Chinese hamster ovary and fibroblast cell lines, both positive and negative results were reported with *p*-methoxybenzaldehyde, *m*-methoxy-*p*-hydroxybenzaldehyde, methyl 2-hydroxybenzoate, benzyl alcohol, and benzaldehyde, with the positive result usually occurring at the higher culture concentrations [Kawachi *et al*., 1980a, 1980b; Kasamaki *et al*., 1982; Ishidate *et al.*, 1984; Sofuni *et al*., 1985; Kasamaki and Urasawa, 1985; Galloway *et al*., 1987; Jansson and Zech, 1987; NTP, 1989]. Negative results were reported for benzyl acetate in the ABS [Galloway *et al*., 1987; Matsuoka *et al*., 1996] whereas positive results were reported for sodium benzoate [Kawachi *et al*., 1980a, 1980b; Ishidate *et al.,* 1984]. Weak positive results were reported for benzoic acid in the ABS assay [Ishidate *et al*., 1984]. The positive results in the ABS assays were generally obtained independently of the presence or absence of metabolic activation.

The authors of the MLA and ABS assays [Heck *et al*., 1989] have emphasized that the positive results in the MLA and ABS assays may be artifacts resulting from changes in culture pH and osmolality. Treatment with high dose levels of substances (*e.g*., reactive aldehydes and carboxylic acids) with the potential to alter acidity or osmolality may induce a significant increase in mutant frequencies or aberrations in these assays. Often the results are inconsistent with the results of other genotoxicity assays (*i.e.*, AMS and UDS) [Heck *et al*., 1989].

In the SCE assay, equivocal results were reported for *p*-methoxybenzaldehyde, m-methoxy-p-hydroxybenzaldehyde, benzyl alcohol and benzaldehyde, in Chinese hamster ovary cell lines and in human lymphocytes [Galloway *et al*., 1987; Sasaki *et al*., 1987, 1989; Jansson *et al*., 1986, 1988; Jansson and Zech, 1987; NTP, 1989]. Negative results were obtained in this assay for benzyl acetate, methyl 2-hydroxybenzoate, sodium benzoate, and benzoic acid [Kawachi *et al.,* 1980a, 1980b; Galloway *et al.,* 1987; Jansson *et al.*, 1988].

No UDS was observed in rat hepatocytes exposed to benzaldehyde, m-methoxy-p-hydroxybenzaldehyde, or benzyl acetate [Mirsalis *et al*., 1983; Heck *et al*., 1989].

In summary, data available for more than 100 *in vitro* genotoxicity assays for 9 members of the category and five metabolic precursors or metabolites of benzyl derivatives indicate a low genotoxic potential for members of this chemical category.

3.4.2.2 In Vivo

In vivo mutagenicity and genotoxicity data exist for 4 of the substances (benzyl acetate, *m*-methoxy-*p*-hydroxybenzaldehyde, methyl 2-hydroxybenzoate, and benzaldehyde) in this group and for 5 structurally related substances (benzyl alcohol, ethyl m-methoxy-p-hydroxybenzaldehyde, sodium 2-hydroxybenzoate, sodium benzoate, and *p*-ethoxybenzaldehyde).

None of these substances showed any evidence of genotoxicity in well-recognized *in vivo* assays (mouse micronucleus, sex-linked recessive lethal, and *in vivo-in vitro* UDS assays). In mammals, compounds were administered orally, by gavage, or by intraperitoneal injection at doses that were significant fractions of the reported lethal dose levels. With the exception of sodium 2-hydroxybenzoate administered at high dose level (Saglo, 1982), other substances in the category were negative in rat chromosomal aberration tests.

Benzyl acetate produced negative findings in micronuclei, and UDS [Mirsalis *et al*., 1989; Shelby *et al*., 1993; Steinmetz and Mirsalis, 1984]. Ethyl vanillin (*m*-ethoxy-*p*-hydroxybenzaldehyde) and sodium benzoate produced negative results in chromosomal aberrations in mice and rats, respectively [Kawachi *et al*., 1980a, 1980b; Furukawa *et al.,* 1989], whereas sodium 2-hydroxybenzoate (sodium salicylate) induced chromosomal aberrations in rats but only at high dose levels [Saglo, 1982]. The micronucleus test also was negative with benzyl alcohol, *m*-methoxy-*p*-hydroxy benzaldehyde, ethyl vanillin, and *p*-ethoxybenzaldehyde [Wild *et al.,* 1983; Hayashi *et al.*, 1988; Inouye *et al.*, 1988; Furukawa *et al.*, 1989].

In the (sub-mammalian) sex-linked recessive lethal mutation assay in fruit flies (*Drosophila melanogaster*), negative results were obtained with benzyl alcohol, benzaldehyde, benzyl acetate, ethyl vanillin, and *p*-ethoxybenzaldehyde, either after feeding or administration by intraperitoneal injection [Wild *et al.,* 1983; Woodruff *et al*., 1985; Foureman *et al*., 1994]. In the silkworm, sodium benzoate exhibited no evidence of mutagenicity at any test concentration (Kawachi *et al.*, 1980b).

Given that the *in vitro* and *in vivo* results both consistently demonstrate that the substances exhibit a low order of genotoxic potential, no additional studies are required for members of this chemical category.

3.4.3 Repeat Dose Toxicity

The National Toxicology Program (NTP), as part of an extensive testing regime, has conducted subacute, subchronic and carcinogenicity studies in rats and mice on 2 of the substances in this group (benzyl acetate tested twice, once *via* administration by gavage and once by microencapsulated in the diet and benzaldehyde by gavage) and one (1) structural relative (benzyl alcohol). Based on extensive metabolic data that benzyl acetate is readily hydrolyzed to benzyl alcohol *in vivo*, and benzyl alcohol is rapidly oxidized to benzaldehyde *in vivo*, the results of all the NTP studies are reviewed together in the appropriate sections below.

In addition to the NTP work, subacute studies are available for 1 category member (benzyl benzoate) and longer-term studies are available for 5 other members of this group (*p*-methoxybenzaldehyde, benzyl benzoate, *m*-methoxy-*p*-hydroxybenzaldehyde, methyl benzoate, methyl 2-hydroxybenzoate, and pentyl 2-hydroxybenzoate), as well as 2 structurally related substances (benzoic acid and sodium benzoate).

Overall, numerous repeat-dose studies using various routes of exposure have been conducted in different animal species with members of this chemical category or their close structural relatives, as discussed below. It is important to note that all the benzyl derivatives in this category are eventually metabolized to a common metabolite, benzoic acid, and are rapidly excreted in the urine as benzoic acid or as its glycine, sulfate, or glucuronic acid conjugate. For this reason, the repeat-dose studies currently available provide adequate support for the safety of the benzyl derivatives. Moreover, the levels at which no adverse effects were reported were sufficiently high to accommodate any potential differences among the members of the category.

3.4.3.1 Subacute Studies

3.4.3.1.1 Rats

In preliminary dose range-finding studies [NTP, 1986, 1989, 1990], benzaldehyde, benzyl alcohol, and benzyl acetate were administered to rats by corn oil gavage for 14-16 days at doses up to 1,600, 2,000, or 4,000 mg/kg bw/d, respectively. The no-observed-adverse-effect levels (NOAELs) determined from these studies based on increased mortality were 400, 500, and 1,000 mg/kg bw/d for benzaldehyde, benzyl alcohol, and benzyl acetate, respectively.

Benzyl acetate also has been evaluated in a 28-day feeding study at dietary concentrations reaching 50,000 ppm (approximately 5000 mg/kg bw/d) [Abdo *et al.,* 1998]. No effects on mortality or body weight of rats were reported at dietary concentrations up to 20,000 ppm (approximately 2000 mg/kg bw/d) and when a glycine supplement was fed in conjunction with the highest dose, mortality and signs of toxicity were significantly reduced. The authors concluded that adequate levels of glycine were instrumental in reducing the toxicity of benzyl acetate.

In addition to oral exposures, 1 of the substances has been tested in rats through inhalation (benzaldehyde). The 14-day inhalation study [Laham *et al.,* 1991] with benzaldehyde showed overall, mild irritation of the nasal mucosa, effects of the central nervous system, and some hematological and biochemical effects at exposures between 500 and 1,000 ppm was reported. Exposed male rats, but not female rats, showed goblet cell metaplasia thought to be related to adaptation following exposure to benzaldehyde.

3.4.3.1.2 Mice

The preliminary testing conducted by the NTP also included gavage mouse studies with benzyl acetate, benzyl alcohol, and benzaldehyde [NTP, 1986, 1989, 1990]. Mice were administered benzyl acetate, benzyl alcohol, or benzaldehyde for 14-16 days at doses of up to 2,000, 2,000, or 3,200 mg/kg bw/d, respectively. Based on increased mortality, the NOAELs were determined to be 500, 500, and 400 mg/kg bw/d for benzyl acetate, benzyl alcohol, and benzaldehyde, respectively.

3.4.3.1.3 Rabbits

When topically applied to the skin of rabbits for 28 days, methyl 2-hydroxybenzoate produced increased mortality at 4 ml/kg bw/d, skin lesions at 2 ml/kg bw/d, and no effects at 1 ml/kg bw/d or less [Webb and Hansen, 1963].

3.4.3.2 Subchronic Studies

3.4.3.2.1 Rats

When benzyl acetate was administered by gavage for 13 weeks [NTP, 1986], survival and body weights were slightly reduced at the highest dose administered of 1000 mg/kg bw/d. In addition, high-dose males and females from the 2 highest dose groups (500 and 1000 mg/kg bw/d), showed trembling, ataxia, and sluggishness. At necropsy, thickened stomach walls were reported in 2/9 males and 4/10 females in the high-dose group. Based on these results, doses of 250 and 500 mg/kg bw/d were selected for a 2-year study.

Similarly, when benzaldehyde was administered by gavage to rats [NTP, 1990], survival and body weights were reduced at the highest dose tested (800 mg/kg bw/d). Also at the high dose, multiple histopathological effects were reported in the brain, forestomach, liver, and kidney. Based on the various lesions reported at the high dose, the NOAEL was determined to be 400 mg/kg bw/d and doses selected for a 2-year study were 200 and 400 mg/kg bw/d.

In the dietary study [NTP, 1993], benzyl acetate caused an increase in mortality (90%) at the highest concentration tested of 50,000 ppm, and body weights were significantly decreased at 12,500 and 25,000 ppm. At 50,000 ppm, lesions of the brain, kidney, tongue and skeletal muscle were noted in both sexes. Excluding the rats from the high-dose group, the only statistically significant finding in clinical chemistry was lower cholesterol levels in females fed 12,500 or 25,000 ppm benzyl acetate. In a couple of males from the 12,500 and 25,000 ppm groups, testicular atrophy was reported. None of the rats had hepatic lesions. Benzyl acetate, when fed at up to 6,250 ppm (approximately 460 or 480 mg/kg bw/d for males and females, respectively) for a period of 13 weeks produced no effects in rats.

Benzyl alcohol, a structural relative and metabolic intermediate of this group, was associated with decreased survival and body weight at the highest dose tested (800 mg/kg bw/d) when administered by gavage to rats for 13 weeks [NTP, 1989]. High-dose rats staggered, were lethargic and had labored breathing. In addition, histopathologic examination of high-dose rats revealed lesions in the brain, skeletal muscle (males only), thymus (males only), and kidney (males only). The renal lesions were non-specific and similar to age-related renal disease. Based on the effects reported at the high dose, the NOAEL was determined to be 400 mg/kg bw/d.

In addition to the NTP study, benzaldehyde has been tested in 2 other studies [Sporn *et al.,* 1967; Hagan *et al*., 1967] reported with limited experimental detail and prior to the establishment of GLP guidelines. In a study [Sporn *et al.*, 1967], adult white rats were orally administered doses of 10 mg benzaldehyde diluted in 0.1 ml of oil on alternate days for a period of 8 weeks. No hepatic enzyme activity was reported. The authors conducted a second investigation lasting 12 weeks in which no effects on growth, liver or adrenal gland weight were reported. In a study conducted by the FDA, [Hagan *et al.*, 1967] tested benzaldehyde as well as 2 additional members of this group: *p*-methoxybenzaldehyde and *m*-methoxy-*p*-hydroxybenzaldehyde. Each substance was fed to groups of Osborne-Mendel rats at concentrations of 0 or 10,000 ppm (approximately 500 mg/kg bw/d) for 16 weeks or 0 or 1,000 ppm (approximately 50 mg/kg bw/d) for 27-28 weeks. No clinical, hematological or histopathological effects were reported with any of the substances tested.

Groups of male and female Wistar rats were fed diets containing 0, 50, 500, or 5000 ppm pentyl 2-hydroxybenzoate (approximately 0, 4.7, 46, or 415 mg/kg bw/d for males and 0, 4.8, 46.9, or 475 mg/kg bw/d for females, respectively) for a period of 13 weeks [Drake *et al*., 1975]. At the high-dose, both sexes showed statistically significant decreases in body weight gain and food consumption. Water consumption was significantly decreased on day 1 in all animals fed pentyl 2-hydroxybenzoate, but was comparable to controls in treated males and was significantly increased in high-dose females. Approximately half of the high-dose animals showed signs of respiratory infection. Hematology results showed no consistent findings. Urinalysis showed a lower specific gravity of urine from high-dose rats that was only significant (p less than 0.05) at week 6 for both sexes and at week 13 for females. A 6-hour collection taken during week 2 showed females produced an increased urine volume. Organ weights showed sporadic differences from controls, which were not consistently observed at the necropsies conducted at 6 and 13 weeks, or were not dose-related with a few exceptions. The authors suggested that the increased volumes of urine with a lower specific gravity in high-dose females might have been a result of a functional injury to the kidney [not reported in males]; however, this was not supported by histopathological examination of the kidneys. The slight increase in relative kidney weights was not seen at 6 weeks and was not dose-related in the males (only increased at the mid-dose in males and at the high-dose in females) at 13 weeks. Although the authors indicated that this may be a toxic effect on the kidney, with the absence of histopathological changes, no dose-related effect in males and only a minor, although significant, increase in high-dose females, this conclusion is difficult to ascertain. The increased relative liver weight noted in high-dose females was not accompanied with histopathological changes and the authors suggested that the increase may have been related to an increased metabolic demand which was considered to "be of little toxicological significance". Relative spleen weight was significantly increased at the 2 highest doses in male rats at 6 weeks but not at 13 weeks and was only seen in high-dose females at 13 weeks. The increased relative brain weights were to be expected with the reduction in body weight gain, since even in starved animals, brain weight is not affected. In both treated and control rats, histopathology revealed "lung changes consisting of thickening of the alveolar walls together with lymphocyte cuffing of the blood vessels and bronchi." In addition in all groups, there was lymphocyte infiltration and protein exudate in the renal tubules. In a paired-feeding study, groups of rats were fed 0 or 5000 ppm in the diet for 98 days and there was no statistically significant differences in the rate of body weight gain between treated and control animals. The reduction in body weight gain appeared to be due to the poor palatability of the test diet as indicated by the reduced food consumption and supported by the results of the paired-feeding study. Although the authors proposed that the study results indicated that the "no-untoward-effect level for pentyl 2-hydroxybenzoate is 50 ppm of the diet", the inclusion of the increased relative kidney weight in male rats fed 500 ppm as an adverse effect is not realistic, since at the high-dose there was no difference from controls and the effect was not dose-related in male rats. In addition the effects on the spleen reported at 500 ppm in male rats at 6 weeks was not reproduced at 13 weeks. Therefore the reviewer suggests a no-observed-adverse-effect level of 500 ppm based on the effects reported at the highest dose tested.

Methyl 2-hydroxybenzoate, another group member, has been well studied [Webb and Hansen, 1963; Abbott and Harrisson]. In one study [Webb and Hansen, 1963], when rats were administered 0, 0.1 or 1% methyl 2-hydroxybenzoate in the diet (approximately 0, 50, or 500 mg/kg bw/d) over a period of 17 weeks, no gross or histopathological effect were reported, but that highest dose did reduce body weight gain. A series of experiments were conducted to assess the possible effect of methyl 2-hydroxybenzoate on osseous changes in rats [Abbott and Harrisson].

In these experiments, methyl 2-hydroxybenzoate was fed at up to 2% in the diet for up to 30 weeks under various conditions. When fed at 1.13 or 2% in the diet (approximately 565 or 1,000 mg/kg bw/d, respectively), decreased body weight gain as a result of decreased food consumption was reported and, X-rays taken at week 10 showed increased bone density at the metaphyses of the femur, humerus, tibia, and radius. In an 11-week experiment, the feeding of 2% methyl 2-hydroxybenzoate was compared to 2.0% methyl *p*-hydroxybenzoate, 2.0% methyl *m*-hydroxybenzoate, 2.36% 2-acetylbenzoic acid, or 2.1% sodium 2-hydroxybenzoate. Bone lesions (*i.e.,* increased bone density) were reported in rats fed methyl 2-hydroxybenzoate, 2-acetylbenzoic acid, or sodium 2-hydroxybenzoate. No effects were seen in animals fed methyl *p*-hydroxybenzoate or methyl *m*-hydroxybenzoate. The authors concluded that the effect on bone is related to the 2-hydroxybenzoic acid structure (salicylate). The nutritional implications of the reduced food intake and body weight gain were assessed over 6 weeks in pair-fed rats at 0, 0.6, or 2% methyl 2-hydroxybenzoate. Growth curves showed rats consuming 0.6% methyl 2-hydroxybenzoate *ad libitum* were slightly below those not receiving methyl 2-hydroxybenzoate. Mean body weight was reduced in rats fed 2.0% methyl 2-hydroxybenzoate *ad libitum* and mortality reached 90% by the end of the study. In all cases, the pair-fed rats showed similar body weight curves and mortalities to those of rats fed 2.0% methyl 2-hydroxybenzoate *ad libitum*. In another 11-week study, [Abbott and Harrisson] determined a more precise lowest-observed-effect level (LOEL) of 1.2% methyl 2-hydroxybenzoate for the appearance of bone lesions in rats. No bone lesions were observed in rats fed 0.9% methyl 2-hydroxybenzoate or less. A 12-week study was used to determine that supplementation with approximately 3% calcium carbonate helped prevent formation of bone lesions and the reduction of growth and survival in methyl 2-hydroxybenzoate-treated rats.

Another member of this group, *m*-methoxy-*p*-hydroxybenzaldehyde, was assessed in an inhalation study and a feeding study. In the inhalation study, rats were exposed in a chamber to 0.5, 1.5, or 15 mg/m^3 of m-methoxy-p-hydroxybenzaldehyde for 4 months [Mazaruk, 1982]. At the highest exposure, the author reported effects on the nervous and cardiovascular systems, the liver, adrenal glands and hematological parameters. In the feeding study, male rats were fed diets containing 0, 0.1, 0.5, or 1.0% m-methoxy-p-hydroxybenzaldehyde (approximately 0, 40, 214, or 437 mg/kg bw/d, respectively) for a period of 26 weeks [Hazleton Laboratories, 1955]. Mild respiratory infections were reported throughout all test groups, including controls. There were no differences in survival, body weight, food consumption, and pathology between treated rats and controls.

Methyl benzoate administered to rats (route not specified) for 45 days at doses of 111 or 500 mg/kg bw/d, did not produce any histological findings, but was reported to produce some effects on blood parameters [Kravets-Bekker and Ivanova, 1970].

3.4.3.2.2 Mice

When benzyl acetate was administered to B5C3F1 mice by gavage at up to 1,000 (males) or 2,000 (females) mg/kg bw/d in corn oil for 13 weeks, mortality was increased in high-dose females (70%), but not in males [NTP, 1986]. Females administered the highest dose of 2,000 mg/kg bw/d also exhibited trembling, inactivity, labored breathing and depressed body temperature. No compound-related gross or microscopic pathological effects were reported in any of the treated animals. Based on these results, doses of 500 and 1,000 mg/kg bw/d were selected for a 2-year study.

Mortality was also increased (9/10 males and 1/10 females died) when benzaldehyde was administered by corn oil gavage to B5C3F1 mice at the highest dose tested (1,200 mg/kg bw/d) [NTP, 1990]. At 600 mg/kg bw/d, the final mean body weight of males was 9% lower than controls. Mild to moderate renal tubule degeneration occurred in all males in the high-dose group and in 1/10 males in the 600 mg/kg bw/d group. No other compound-related effects were reported. Based on the mild renal lesions and depressed body weight gain, the doses selected for a 2-year study were 300 and 600 mg/kg bw/d.

Benzyl alcohol, a metabolic precursor of benzaldehyde, did not increase mortality at the highest dose tested (800 mg/kg bw/d), but did produce slight decreases in body weight of 5 and 8% in females at 400 and 800 mg/kg bw/d, respectively [NTP, 1989]. At the highest dose level, staggering was seen in both male and female mice during the first 2 weeks of the study. No adverse effects were produced at 200 mg/kg bw/d or lower.

In the 13-week dietary study [NTP, 1993], benzyl acetate significantly reduced final body weights and body weight gains at all doses tested (3,130 to 50,000 ppm in the diet or approximately 425 to 7,900 mg/kg bw/d for males and 650 to 9,400 mg/kg bw/d for females). One death occurred in one mouse of each sex in the high-dose group. Significant differences, secondary to decreased body weights, in absolute and relative brain, kidney, liver, pancreatic, prostatic, seminal vesicle, splenic, testicular and thymus weights were reported in all dose groups. Several female mice from the high-dose group and 1 female each from the 12,500 and 25,000 ppm dose groups had tremors. No other clinical signs were reported. Hematological and clinical chemistry parameters were not affected in either sex. In the high-dose group, brain and liver necrosis were reported in 4 females and 1 male, respectively. No other lesions were reported.

The primary metabolite (benzoic acid) of the substances in this group was administered by gavage at a dose of 80 mg/kg bw/d to male and female crossbred white mice for a period of 3 months [Shtenberg and Ignat'ev, 1970]. Weight gain of the treated animals was reduced compared to control animals and was not considered to be related to reduced feed intake, but it may have been due to external stress factors.

3.4.3.2.3 Rabbits

Benzyl benzoate was dermally applied to a total of 441 rabbits at various unspecified doses [Draize *et al.,* 1948]. Pathology was conducted on these animals at termination and a 90-day LD50 value of 2.0 ml/kg bw (>2000 mg/kg bw/d) was calculated from a dosage-mortality curve. In addition, atrophy of testis was reported in survivors at the 2 highest dose levels.

3.4.3.2.4 Dogs

Male and female beagle dogs were orally administered a capsule containing up to 1,200 mgkg/d/6 days/week for 59 days of methyl 2-hydroxybenzoate [Webb and Hansen, 1963]. The dogs in the 3 highest dose groups (500, 800, and 1,200 mg/kg/d) died within one month of treatment or were killed *in extremis*. Except for fatty liver in one dog each of the 2 highest dose groups, no other microscopic effects were reported. Doses of 500 mg/kg bw/d or higher in dogs are not well tolerated and reported to cause fatty liver, diarrhea, vomiting and eventual death. No adverse effects were reported at doses up to 250 mg/kg bw/d over 59 days.

In two subchronic studies, beagle dogs were administered methyl 2-hydroxybenzoate [Abbott and Harrisson]. In the first study, male and female beagle dogs were administered up to 800 mg/kg bw/d of methyl 2-hydroxybenzoate in 2 divided doses by capsule, 6 days/week for up to 7.5 months. Survival was reduced in the 2 highest dose groups (500 and 800 mg/kg bw/d) to 67 and 0%, respectively. Body weight was not affected in the 2 lowest dose groups (150 and 300 mg/kg bw/d) and 1 dog given 500 mg/kg bw/d showed a slight loss in body weight. Hematological analyses and urinalyses conducted on control dogs and dogs from the 2 lowest dose groups compared favorably. At 6.5 months, dogs administered 300 mg/kg bw/d of methyl 2-hydroxybenzoate discontinued treatment and were observed an additional 1.5 months. Overall, there were no gross findings and the only organ weight findings were dose-related increases in absolute and relative liver and kidney weights of all treated dogs (excluding dogs from the 300 mg/kg bw/d dose group that discontinued treatment). The enlargement of the liver and kidneys was not accompanied by histopathological changes with the exception of extremely subtle changes in the liver indicative of increase in liver cell size and cytoplasmic granularity, but not hepatotoxicity.

In the follow-up study, Beagle dogs of both sexes were orally administered up to 167 mg/kg bw/d of methyl 2-hydroxybenzoate or 2-acetylbenzoic acid (aspirin) by capsule in 2 divided for a period of 6 months [Abbott and Harrisson]. Four dogs from the high-dose and control groups were discontinued from treatment at 6 months and were maintained on basal diets for an additional 2 months. Survival to the end of the study was 100%.

Many dogs in the test groups developed signs of seborrhea oleosum and pyoderma after 2 months, which was corrected with the addition of lard to the diet. Hematological analyses were comparable among treatment and control groups. At the 6-month necropsy, the only compound-related gross observations were changes in the gastric mucosa including gastric hemorrhage in dogs given the highest dose of ASA. Histopathological examination and relative weights of the liver and kidneys showed no differences between treated and control dogs.

Another member of this group, *m*-methoxy-*p*-hydroxybenzaldehyde, was evaluated in a 26-week oral toxicity study, in which male and female dogs were administered 0, 25, or 100 mg/kg bw/d of *m*-methoxy-*p*-hydroxybenzaldehyde by capsule, 5 days/week [Hazleton Laboratories, 1955]. No effects on blood parameters, urinalysis, or histopathology were reported.

3.4.3.2.5 Humans

Six clinicians conduced a retrospective evaluation of young actively growing children treated with 2-hydroxybenzoate (salicylates) for various forms of juvenile rheumatoid arthritis [Abbott and Harrisson]. Daily 2-hydroxybenzoate doses ranged from 100 to 3,240 mg and treatment ranged from several months to 14 years. Periodic X-rays were available for evaluating any possible bone lesions. One hundred and fifty-five (155) cases were assessed and there was no indication that prolonged salicylate therapy causes bone lesions in children.

A similar study was conducted to evaluate a possible association between large daily doses of 2-hydroxybenzoates (salicylates) and hepatomegaly in children [Abbott and Harrisson]. Two hundred and eighteen (218) case studies in which up to 4,800 mg/d for periods of up to 10 years were evaluated and it was concluded that salicylate therapy does not cause hepatomegaly or effect growth or weight gain. Hepatomegaly was noted occasionally prior to treatment or during the course of treatment, but was considered a "concurrent happening".

3.4.3.3 Chronic Studies

3.4.3.3.1 Rats

Benzyl alcohol, benzaldehyde and benzyl acetate were administered in corn oil gavage five days per week to groups of 50 F344/N rats or B6C3F1 mice for a period of 2 years at 2 dose levels [NTP, 1986, 1989, 1990]. An additional study was conducted in which test groups of 60 rats or mice were maintained on diets containing benzyl acetate [NTP, 1993]. Animals were observed once weekly for 12-13 weeks and at least monthly thereafter. All animals were subject to necropsy after death, or at the end of the study. Throughout these studies, mean body weights were comparable among all groups, except for rats and mice maintained on the high dose level of benzyl acetate in the feed, which was reduced (5 to 16%) due to decreased feed consumption. Increased mortality seen in some of the groups was attributable to the gavage procedure, reflux and aspiration of the gavage material into the lungs, or administration errors resulting in direct disposition of material into the lungs. Summaries of the rat studies and results are described below and the results of the mouse studies are discussed in the section on mice.

3.4.3.3.1.1 Benzyl Alcohol

Groups of male and female F344/N rats were administered 0, 200, or 400 mg/kg bw/d, 5 days/week of benzyl alcohol, in corn oil by gavage for 2 years. Survival was significantly decreased in high-dose females after week 50 and in low-dose females after week 71. In the lung, there was an increase in the incidence of hemorrhage and foreign material in treated rats dying before study termination possibly as a result of the gavage procedure. In females, the incidence of anterior pituitary gland neoplasms decreased with increasing dose. The NTP concluded that benzyl alcohol produced "no evidence of carcinogenic activity" in this study [NTP, 1989].

3.4.3.3.1.2 Benzaldehyde

Groups of 50 male and 50 female F344/N rats were administered 0, 200, or 400 mg/kg bw/d, 5 days/week, of benzaldehyde in corn oil by gavage for 2 years. Survival was significantly decreased in the high-dose male group after day 373; however, there were no other compound-related effects in any of the rats. The NTP concluded that there was “no evidence of carcinogenic activity” in rats given benzaldehyde under these study conditions [NTP, 1990].

3.4.3.3.1.3 Benzyl Acetate

Groups of 50 male and 50 female F344/N rats were administered 0, 250, or 500 mg/kg bw/d, 5 days/week, of benzyl acetate in corn oil by gavage for 2 years [NTP, 1986]. There were no effects on body weight or survival and there were no clinical effects observed. There was a statistically significant (p less than 0.05) increase in the incidence of preputial gland tumors in males; however, NTP did not associate this with benzyl acetate administration since the combined incidence of adenomas, adenocarcinomas, or carcinomas was not increased. There was also a statistically significant increase in the incidence of pancreatic acinar cell adenomas in males of the high-dose group (p less than 0.007) with tumors often multiple in rats. The incidence of the multiple pancreatic acinar cell adenomas in the control, mid- and high-dose male rats was 20 (10/50), 24 (12/50), and 45% (22/49). The NTP concluded, “under conditions of the gavage studies, benzyl acetate administration was associated with increased incidence of acinar cell adenoma of the exocrine pancreas in male F344/N rats; the gavage vehicle may have been a contributing factor. No evidence of carcinogenicity was found for female F344/N rats.”

In a subsequent dietary study [NTP, 1993], F344/N rats were maintained on diets containing 0, 3,000, 6000, or 12,000 ppm benzyl acetate (approximately 0, 130, 260, or 510 mg/kg bw/d and 0, 145, 290, or 575 mg/kg bw/d for male and female rats, respectively) for 103 weeks. There was no effect on survival, hematology, or clinical chemistry. Mean body weights and food consumption were slightly reduced in the high-dose groups. No treatment-related changes in the incidence of neoplastic and non-neoplastic lesions were reported. Therefore, the NTP concluded, “under conditions of these 2-year feeding studies, there was no evidence of carcinogenic activity of benzyl acetate in male or female F344/N rats receiving 3,000, 6,000, or 12,000 ppm.”

Upon completion of the second study, the NTP concluded that the increased incidence of pancreatic acinar cell neoplasms specific to male rats reported in the earlier study was probably due to the use of corn oil as a vehicle in the gavage study. Administration of high levels of fat to experimental animals has been shown to enhance the development of spontaneous and chemical-induced neoplasms [NTP, 1994a]. More specifically, administration of corn oil and other high-fat vehicles by gavage daily at doses of 2.5, 5, or 10 ml/kg bw for 2 years was associated with increased incidence of proliferative lesions of the exocrine pancreas [NTP, 1994b].

In addition to the NTP studies, benzyl acetate was evaluated for its potential to promote pancreatic carcinogenesis in male F344 rats in 3 separate experiments [Longnecker *et al.,* 1990]. Rats were intraperitoneally injected twice with 30 mg/kg bw of azaserine (pancreatic carcinogen) at 16 and 23 days of age and fed up to 0.8% benzyl acetate in the diet (approximately 400 mg/kg body weight/d) for a period of 6 months (experiment 1) or 1 year (experiment 2). In experiment 3, rats received 0 or 0.8% benzyl acetate in the diet for a 2-year period without the azaserine injections. In experiment 1, examination of the pancreas showed fewer lesions per cm^3 in rats fed benzyl acetate; however, the mean diameter and the volume percentage of foci were significantly greater in benzyl acetate-treated rats than in controls. Animals in experiment 2 did not survive treatment due to azaserine-induced nephrotoxicity. A marginal statistically significant increase in the incidence of pancreatic carcinoma *in situ* among rats fed 0.8% benzyl acetate in experiment 3 was reported. The authors concluded that benzyl acetate was a weak promoter, but not an initiator of pancreatic carcinogenesis in the rat.

In a previous 4-month study [Longnecker *et al.,* 1986], no pancreatic tumor effects were reported in male F344 and Lewis rats administered 500 mg/kg bw/d, 5 days/week of benzyl acetate by gavage or at 0.9% in the diet (approximately 450 mg/kg bw/d).

Two long-term rat studies were conducted in order to assess the effect of methyl 2-hydroxybenzoate on bone. In the first study, groups of Osborne-Mendel rats were fed diets containing up to 2.0% methyl 2-hydroxybenzoate (approximately 1,000 mg/kg bw/d) for 2 years. Rats in the highest dose group (1,000 mg/kg bw/d) exhibited increased amounts of cancellous bone tissue and fewer osteoclasts compared to controls and did not survive past day 49 of the study. At the next 2 highest doses (250 and 500 mg/kg bw/d), a slight excess of cancellous bone was reported in 1/11 and 2/11 bones examined, respectively. Growth inhibition reached statistical significance at the 2 highest dose levels. No other significant effects were reported at any dose [Webb and Hansen, 1963].

A second, supplemental study was conducted to examine the reported changes in bone of Osborne-Mendel rats fed methyl 2-hydroxybenzoate in the diet at 2% (approximately 1,000 mg/kg bw/d). Osborne-Mendel rats of both sexes were fed 2% methyl 2-hydroxybenzoate in the diet for a period of 71 days. Methyl 2-hydroxybenzoate at 2% in the diet of rats was reported to produce increased mortality, changes in bone, and effects on lung and stomach tissue [Webb and Hansen, 1963]. However, the individual data for these results were not available and could not be fully interpreted.

m-Methoxy-*p*-hydroxybenzaldehyde also has undergone long-term testing in rats. Osborne-Mendel rats were fed up to 20,000 ppm *m*-methoxy-*p*-hydroxybenzaldehyde in the diet for 2 years or up to 50,000 ppm in the diet for 1 year [Hagan *et al.*, 1967]. Clinical, hematological, gross, and histopathological examinations revealed no adverse effects.

White and gray rats were administered 0.005 or 0.05 mg/kg bw/d of methyl benzoate for 6 months [Kravets-Bekker and Ivanova, 1970]. The general condition of the animals in both dose groups did not differ from controls. At the high dose, there was decrease in the number of reticulocytes (p less than 0.01) but there was no difference from controls in prothrombin time or phagocytic activity at either dose. In behavioral tests, at the high dose, the latent period for response to "bell" or "light" stimulus was increased. Also, there was an increase in the number of sulfhydryl groups in cerebral tissue of high-dose rats. At necropsy, congestion and swelling of the hepatic central veins and capillaries was reported in high-dose rats. There were no histological findings in the low-dose animals.

Two structural representatives of this group, benzoic acid and sodium benzoate, also have been tested in long-term studies. In an 18-month study, male and female Wistar rats were fed 40 mg/kg bw/d of benzoic acid in a paste prior to normal feeding [Shtenberg and Ignat'ev, 1970]. Similarly, male and female Wistar rats were fed 40 mg/kg bw/d of benzoic acid in conjunction with 80 mg/kg bw/d of sodium bisulphite. The results were not clearly reported; however, it appeared that survival was only decreased in rats fed the benzoic acid/sodium bisulphite combination and that the benzoic acid/sodium bisulphite combination-fed rats showed more of an effect in the stress tests, had increased erythrocyte sedimentation rates, and a decreased level of blood ketones compared to controls. No effects on blood alkalinity, C-reactive protein levels and blood morphology were reported in the treated rats.

Male and female Fischer 344 rats were fed 0, 1 or 2% sodium benzoate in the diet (approximately 0, 370, or 735 mg/kg bw/d for males and 0, 445 or 880 mg/kg bw/d for females, respectively) for a period of 18 to 24 months [Sodemoto and Enomoto, 1980]. Mortality of all groups was affected by hemorrhagic pneumonia; however, there was no reported difference in mortality, growth or food intake between treated and control rats. There also was no significant difference in benign and malignant tumors in treated rats compared to controls.

3.4.3.3.2 Mice

Summaries of the mouse studies conducted by the NTP and results are described below.

3.4.3.3.2.1 Benzyl Alcohol

Groups of male and female B6C3F1 mice were administered 0,100, or 200 mg/kg bw/d, 5 days/week of benzyl alcohol in corn oil by gavage for 2 years. In males, the incidence of Harderian gland adenomas tended to decrease with increasing dose. Male mice in the high-dose group showed a slight increase in the incidence of adrenal cortex adenomas; however, the NTP did not consider this compound related. The NTP concluded that benzyl alcohol produced "no evidence of carcinogenic activity" in this study [NTP, 1989].

3.4.3.3.2.2 Benzaldehyde

Groups of 50 male B6C3F1 mice were administered 0, 200, or 400 mg/kg bw/d, 5 days/week of benzaldehyde in corn oil by gavage for 2 years. Groups of 50 female mice were administered 0, 300, or 600 mg/kg bw/d of benzaldehyde. There were no compound-related clinical signs or effects on body weight, and survival was not affected by treatment. A non-statistically significant increase in the incidence of forestomach focal hyperplasia was reported in both sexes. An increase in the incidence of squamous cell papillomas of the forestomach was reported in both sexes at all doses tested and reached statistical significance in the female mice. There was no increase in the incidence of squamous cell carcinomas of the forestomach in either sex. The NTP considered the increase in forestomach papillomas to be attributable to a concurrent increase in hyperplasia as a result of benzaldehyde treatment and, therefore, concluded that there was "some evidence of carcinogenic activity" in mice under these study conditions [NTP, 1990].

The occurrence of squamous cell papillomas and forestomach hyperplasia in rodents is common in NTP bioassay gavage studies in which a high concentration of an irritating material in corn oil is delivered daily by needle into the forestomach for two years. High concentrations of aldehydes such as malonaldehyde, furfural, and benzaldehyde [NTP 1988, 1990, 1993] and other irritating substances including dihydrocoumarin and courmarin [NTP 1990, 1992] delivered in corn oil by gavage are consistently associated with these phenomena in the forestomach of rodents. Squamous cell papillomas are benign lesion of surfaces covered with squamous epithelium. A majority of papillomas arise as a result of chronic irritation, or from infection from some strains of viruses [Smith and Ford, 1993]. Additionally, forestomach hyperplasia and papillary proliferation in these studies did not progress to squamous cell carcinomas.

Apparently, the combination of daily introduction of a dosing needle into the forestomach and delivery of a high concentrations of an irritating test material in corn oil, which itself is a mild irritant and mitogen, was the likely source of the papillomas in the rodent forestomach. This conclusion is supported by the observation that the occurrence of squamous cell papillomas and forestomach hyperplasia in gavage administration of a test material in corn oil for 2 years (see below; NTP, 1986) disappear when the same substance is administered at similar intake levels in the diet (see below; NTP, 1993). Therefore, the appearance of these benign lesions in the 2-year rodent bioassay have no relevance to humans, given that human exposure occurs when low levels of benzaldehyde are consumed in the diet.

3.4.3.3.2.3 Benzyl Acetate

Groups of 50 male and 50 female B6C3F1 mice were administered 0, 500, or 1,000 mg/kg bw/d, 5 days/week of benzyl acetate in corn oil by gavage for 2 years [NTP, 1986]. Survival in the high-dose female mice showed a statistically significant increase (p=0.005). There were no adverse clinical signs. Body weights treated female mice were slightly higher than controls after week 20. The incidence of hepatocellular adenomas was increased in males (0, 10, and 26%, respectively) and females (0, 0, and 12%, respectively) and reached statistical significance at the highest dose (males, p less than 0.001; females, p less than 0.05). There was no effect on the incidence of hepatocellular carcinomas. Also in males and high-dose females, there was a statistically significant (p less than 0.005) increase in the occurrence of forestomach hyperplasia. Although there was a positive trend for an increased incidence of combined forestomach squamous cell papillomas and carcinomas in both sexes, it did not reach statistical significance. The NTP concluded, "For male and female B6C3F1 mice there was evidence of carcinogenicity, in that benzyl acetate cause increased incidences of hepatocellular neoplasms and squamous cell neoplasms of the forestomach."

In a subsequent dietary study [NTP, 1993], B6C3F1 mice were fed benzyl acetate in the diet at concentrations of 0, 330, 1,000, or 3,000 ppm (approximately and 0, 35, 110, or 345 mg/kg bw/d and 0, 40, 130, or 375 mg/kg bw/d for male and female mice, respectively). Survival was similar to controls with the exception of the high-dose females, which was significantly higher than the control group. Mean body weights were generally 2 to 14% lower than controls (statistical significance not reported). There were no biologically significant changes in hematology or clinical chemistry parameters. Notable was the lack of an increase in neoplasm incidences, particularly hepatocellular and forestomach neoplasm, as was reported in the gavage studies. A dose-related increase in the incidence or severity of non-neoplastic nasal lesions (*i.e.*, mucosa atrophy and degeneration, cystic hyperplasia of the submucosal gland, and luminal exudates and pigmentation of the mucosal epithelium) was reported. NTP considered that the nasal lesions were likely a result of irritation from benzyl acetate vapors and concluded that "there was no evidence of carcinogenic activity of benzyl acetate in male or female B6C3F1 mice" under the conditions of this study.

The increased incidence of hepatocellular adenomas observed in the gavage study was not observed in the dietary study. The NTP proposed that the difference was due to the different mode of administration and the resulting higher plasma levels of benzyl acetate metabolites (benzoic acid) in the gavage study [NTP, 1993]. In a comparative toxicokinetic study of gavage and dietary mode of administration discussed earlier [Yuan *et al.*, 1995], peak plasma concentrations of benzoic acid were higher in the gavage study than those in the dietary study. Although daily dietary intake level and gavage dose levels were similar, gavage administration saturated the benzoic acid elimination pathway. Hippuric acid plasma concentrations were similar indicating depletion of the glycine pool in the gavage study with concomitant increases in free plasma benzoic acid. The authors suggested that higher plasma levels of benzyl acetate and free benzoic acid in the gavage study might be, in part, associated with the different toxicological outcomes of the gavage and dietary studies. There is evidence that the incidence of hepatocellular adenomas in the gavage study were the result of chronic exposure to high levels of the corn oil vehicle.

Statistical analysis of liver adenomas is suspect because the vehicle control animals had aberrantly low incidence when compared to historical controls. Control values for liver adenomas in NTP bioassays vary from 20% to as high as 42%. In this study, the liver adenoma rates were zero. If these historical controls are adopted for analysis, the significance of the adenoma finding in the high-dose animals disappears or becomes marginal ($p = 0.038$ for females, and $p = 0.078$ for males). Procedural concerns include the fact that the diets were not tested for contaminants and there are interpretive problems with corn oil gavage and tumor occurrence [NTP, 1986; Bernard, 1983].

In conclusion, evidence of carcinogenicity in the gavage benzaldehyde and benzyl acetate studies are associated with the repeated gavage administration of high dose levels of test substance in a corn oil vehicle or a statistical anomaly. The above observations strongly suggest that the results of the gavage NTP studies have no significance to humans.

This conclusion is further supported by the lack of tumorigenicity in a chronic studies conducted with a structural representative of this group, sodium benzoate, and the primary metabolite, benzoic acid. No effect on tumor incidence or survival was seen in male and female albino Swiss mice administered 2% sodium benzoate in drinking water (approximately 4,000 mg/kg bw/d) for their life span (up to approximately 112 weeks) [Toth, 1984]. The only reported finding in a 17-month study in which mice were orally administered 40 mg/kg bw/d of benzoic acid was increased survival as compared to controls [Shtenberg and Ignat'ev, 1970].

3.4.3.3.3 Dogs

Only 1 member (methyl 2-hydroxybenzoate) of this group has been tested chronically in dogs. Male and female beagles were orally administered 0, 50, 150, or 350 mg/kg bw/d by capsules, 6 days/week of methyl 2-hydroxybenzoate for 2 years [Webb and Hansen, 1963]. One high-dose female was replaced due to a hepatitis infection after 33 days. Retarded growth and enlarged liver were reported at the 2 highest doses. No effects were reported on hematology. Doses of 50 mg/kg bw/d of methyl 2-hydroxybenzoate or less in dogs over a period of 2 years showed no adverse effects.

3.4.4 Reproductive Toxicity

Studies examining possible reproductive toxicity are available on 3 representative substances (benzyl acetate, benzaldehyde, and methyl 2-hydroxybenzoate) from this chemical category, and the primary metabolite of benzyl esters and benzaldehyde (benzoic acid).

Overall, several reproductive toxicity studies have been conducted with representatives of this group and produced no evidence of reproductive toxicity (see below). As was discussed with the repeat-dose studies, the benzyl derivatives generally follow the similar metabolic pathways and the studies conducted provide an adequate database for this endpoint. In addition, the dose levels tested provide margins of safety large enough to accommodate any differences among the group.

3.4.4.1 Rats

In one study, 2 mg benzaldehyde was administered by gavage to 10 breeding age rats every other day (approximately 5 mg/kg bw/d) for a period of 32 weeks [Sporn *et al.*, 1967]. Two pregnancies per rat were studied, one at 75 days and one at 180 days. There was no statistical significant difference between treatment and control groups. It was reported that fewer females in the treated group became pregnant; however, no data or statistical analyses were performed, and the authors concluded that treatment did not cause a significant change in any of the reproductive parameters measured.

As part of the NTP testing program, sperm morphology and vaginal cytology examinations (SMVCE) were conducted on male and female rats from the benzyl acetate 13-week dietary study [NTP, 1993] and used as a screen for reproductive toxicants [Morrissey *et al*., 1988]. There was no effect on the estrus cycle, sperm motility, density or percent abnormality in any of the doses tested. There was no statistically significant change in the weights of the epididymis, the caudia epididymis, or the testis.

Methyl 2-hydroxybenzoate has been tested in 3-generation studies. In one 3-generation study, male and female Osborne-Mendell rats were fed up to 5,000 ppm methyl 2-hydroxybenzoate in the diet (approximately 250 mg/kg bw/d) 100 days prior to mating [Collins *et al.*, 1971]. The parental rats were mated twice to produce 2 litters: F_{1a} and F_{1b} generations. From these, F_2 and F_3 generations were produced. There was no effect on fertility index at any dose. In the F_1 generation at the 2 highest dietary concentrations (3,000 and 5,000 ppm), there was a decrease in average litter size, survival and in average number of live-born per female. External examination showed no gross abnormalities. The statistically significant changes reported in the F_1 generation were not reported in the F_2 generation, although there was a decreasing trend observed. There were no visible abnormalities and necropsies of the F_2 generation showed no effects. A supplemental study using F_{2b} rats was conducted to test the efficacy of calcium on any adverse effects produced by methyl 2-hydroxybenzoate. Rats from each dose level were co-administered 1,500 ppm calcium carbonate (approximately 600 ppm available as calcium) and were mated and the 1st and 2nd litters were observed as previously described. Supplementation with calcium did not appear to alleviate or enhance the reported effects.

In another 3-generation reproduction study, groups of male and female Wistar rats were fed diets containing up to 0.5% methyl 2-hydroxybenzoate for 60 days after which rats were mated to produce F_1 litters [Abbott and Harrisson]. At about 4 months of age, male and female F_1 rats were randomly selected to produce F_2 litters. No gross abnormalities were reported in any of the litters. No statistically significant differences among treated and control animals were reported for any reproductive parameters examined including mating performance, reproductive performance, number of stillborn, viability, mean litter size, number born, number live-born, and number live at 5 days.

3.4.4.2 Mice

Similar to the assay conducted in rats, SMVCE examinations were performed [Morrissey *et al.*, 1988] on mice at the end of the benzyl acetate 13-week study [NTP, 1993]. With the exception of an increased estrous cycle of the high-dose female mouse group compared to controls accompanied by a decrease in body weight, benzyl acetate had no effect on any of the parameters measured.

In a 3-generation reproduction study, groups of male and female mice were fed diets containing up to 0.5% methyl 2-hydroxybenzoate for 30 days after which mice were mated to produce F_1 litters [Abbott and Harrisson]. At about 4 months of age, F_1 mice were randomly selected to produce F_2 litters. No gross abnormalities were reported in any of the litters. No statistically significant differences among treated and control animals were reported for any reproductive parameters examined including mating performance, reproductive performance, number of stillborn, viability, mean litter size, number born, number live born, and number live at 5 days.

Methyl 2-hydroxybenzoate was tested by the NTP using a Fertility Assessment by Continuous Breeding (FACB) in CD-1 mice [NTP, 1984a, 1984b]. In the initial study groups of male and female mice (F_0 generation) were administered 0, 25, 50 or 100 mg/kg bw/d of methyl 2-hydroxybenzoate in corn oil by gavage during a 7-day premating period, throughout a 98-day cohabitation period and 21-day segregation periods. Methyl 2-hydroxybenzoate administration had no effect on the number of pairs able to produce at least one litter, the number of litters produced per pair, the number of live pups per litter, or the proportion or sex of pups born alive. As a continuation of this study, 1 or 2 female and male pups (F_1 generation) weaned from control and high-dose mice were randomly selected and when they were approximately 90 days of age, they were mated to produce an F_2 generation. Overall, there were no statistically significant effects on mating behavior, fertility rate, or reproductive performance and it was concluded that methyl 2-hydroxybenzoate was not a reproductive toxicant at doses of up to 100 mg/kg bw/d. [NTP, 1984a].

In a second study based on a dose range-finding study, groups of male and female CD-1 mice were administered 100, 250 or 500 mg/kg bw/d of methyl 2-hydroxybenzoate during a 7-day premating period and throughout a 100-day cohabitation period. Controls were administered vehicle only. There were no compound-related deaths nor were there any clinical signs of toxicity. Body weights were not affected by treatment. The fertility index was similar in all groups and ranged from 95 to 100%. At the highest dose, there was a significant decrease (p less than 0.05) in the mean number of litters, the average number of pups/litter, the proportion of pups born alive, and mean live pup weights. In a crossover mating trial, high-dose males were mated with control females, high-dose females were mated with control males and controls were mated with controls. Since there was an unusually low fertility index for controls, the trial was repeated. All groups, including controls, had similar, yet poor, fertility. Other parameters measured (*i.e.,* number of live pups/litter, proportion of pups born alive, sex of pups born alive, and live pup weights were assessed yet the authors could not determine which sex was affected by methyl 2-hydroxybenzoate treatment, but concluded that methyl 2-hydroxybenzoate "does interfere with reproduction in CD-1 mice" without further elaboration. [NTP, 1984b].

The metabolite, benzoic acid, orally administered at doses of 40 mg/kg bw/d to male and female mice for 5 generations as part of a chronic feeding study had no effect on reproduction [Shtenberg and Ignat'ev, 1970].

Given the lack of any significant reproductive effects in these studies, the lack of any toxic effects to male or female reproductive organs in chronic and subchronic studies, and the low toxic and carcinogenic potential of members of this chemical category, no additional studies are recommended for members of this category.

3.4.5 Developmental Toxicity

Possible developmental toxicity has been tested in 2 members of this group (benzyl acetate and benzyl benzoate) and 2 structurally related compounds (benzyl alcohol and sodium benzoate).

Overall, 4 representative substances from this group were tested for developmental toxicity with uniform results, and indicated no teratogenic potential in the absence of maternal toxicity. Again, the representative substances undergo similar metabolism to the entire benzyl derivative group and therefore, provide an adequate representation for this endpoint.

3.4.5.1 Rats

Groups of pregnant Wistar rats were administered up to 1,000 mg/kg bw/d of benzyl acetate by gavage during gestation days 6-15 [Ishiguro *et al.,* 1993]. Controls received vehicle only. Maternal mortality, body weight, food consumption, and clinical and gross examinations were comparable for all groups. On gestation day 20, pregnancies were terminated and there was no reported difference in the number of *corpora lutea*, implantations, live/dead fetuses, or resorptions, implantation ratio, sex ratio, or placenta weight in any treatment group. At the highest dose level, fetal body weight was significantly decreased (p less than 0.05), but was significantly increased in the 2 lowest dose groups (p less than 0.05). There was a statistically significant increase in the combined incidence of organ variations (*i.e.,* slight dilatation of the lateral ventricle and renal pelvis, and presence of levo-umbilical artery) in animals from the 2 highest dose groups. The only skeletal malformation (fused ribs) was in one fetus of the high-dose group, which did not increase the incidence of skeletal malformations compared to controls. Skeletal variations (*i.e.,* wavy ribs, dumbbell shaped vertebrae, absence/splitting of thoracic vertebrae, presence of lumbar ribs and degree of ossification) were statistically increased in the high-dose group. The authors suggested that the skeletal malformations were related to the significant decrease in fetal body weight. No increase in intrauterine death or external variations was noted at any dose level. No adverse effects were seen at or below 500 mg/kg bw/d and the results indicated that benzyl acetate has no teratogenic potential.

Benzyl benzoate was fed to pregnant Wistar rats at concentrations up to 1.0% in the diet during gestation days 0 to 21 [Morita *et al.*, 1980]. There were no effects reported on the fetus relating to external, skeletal or visceral anomalies.

Sodium benzoate administered by oral intubation to pregnant rats during gestation days 6 to 15 at doses reaching 175 mg/kg bw/d produced no effects on maternal or fetal rats [Morgareidge, 1972]. In another teratology study, sodium benzoate fed during gestation only produced adverse effects on the fetus at maternally toxic concentrations in the diet of 4% (approximately 1,600 mg/kg bw/d) or higher [Onodera *et al*., 1978].

3.4.5.2 Mice

In a dose range-finding study, groups of CD-1 mice were gavaged with up to 2,605 mg/kg bw/d of benzyl alcohol during gestation days 6-15 [York *et al.*, 1986]. No control group was used. All animals died at the highest dose and 2 animals died at the second highest dose (1,370 mg/kg bw/d). At 720 mg/kg bw/d, there was no signs of toxicity except for reduced body weight. Based on these results, a dose level of 550 mg/kg bw/d was selected for a teratology study.

In the teratology study, groups of pregnant CD-1 mice were administered 0 or 550 mg/kg bw/d of benzyl alcohol in corn oil by gavage during gestation days 6-15 [York *et al.,* 1986]. Body weight, clinical observations, and mortality were recorded daily throughout treatment and up to 3 days postpartum. All parameters tested, including gestation index, average number of live pups/litter, postnatal survival, and pup body weight, were statistically similar for the treated and control animals.

Groups of CD-1 mice were gavaged with 750 mg/kg bw/d of benzyl alcohol on gestation days 6-13 [Hardin *et al.*, 1987]. Controls received distilled water only. Clinical signs of maternal toxicity were reported and included hunched posture, tremors, inactivity, prostration, hypothermia, ataxia, dyspnea, swollen or cyanotic abdomen, and piloerection. There was no significant difference in maternal body weight measured on days 4 and 7 of gestation between treated and control animals; however, statistically significant decreases were observed in treated females on gestation day 18 and day 3 postpartum. Maternal body weight gain during days 7-18 of gestation was also significantly lower than that of controls. Significant differences were also observed in pup body weight and weight gain, including mean pup weight per litter, mean litter weight change, and mean pup weight change (between day 1 and 3 postpartum). No differences were observed in the mating or gestation indices, the total number of resorptions, the number of live pups per litter, or in pup survival. Eighteen deaths were reported during the treatment period and they were all attributed to the treatment. One more death was reported the day after treatment was terminated. Although the authors concluded that benzyl alcohol was a potential reproductive hazard, the effects observed were in conjunction with significant maternal toxicity.

When administered by oral intubation to pregnant mice at doses up to 175 mg/kg bw/d during gestation days 6 to 15, sodium benzoate did not affect maternal or fetal animals [Morgareidge, 1972].

3.4.5.3 Rabbit

Sodium benzoate administered by oral intubation to pregnant rabbits during gestation days 6 to 18 at doses reaching 250 mg/kg bw/d produced no effects on maternal or fetal rabbits [Morgareidge, 1972].

3.4.5.4 Hamster

Sodium benzoate administered by oral intubation to pregnant hamsters during gestation days 6 to 10 at doses reaching 300 mg/kg bw/d produced no effects on maternal or fetal hamsters [Morgareidge, 1972].

3.4.6 New Testing Required

No human health testing is required for the benzyl derivative category. Numerous genetic, acute, repeat-dose, developmental, and reproductive toxicity studies exist for members of this category, close structural relatives, metabolic precursors, and principal metabolites. Benzyl derivatives are eventually metabolized to a benzoic acid metabolites, either a benzoic acid derivative or 2-hydroxybenzoic acid derivative, that are rapidly excreted in the urine as the glycine, sulfate, or glucuronic acid conjugate. Toxicological data on these substances are directly related to the evaluation members of this category.

Moreover, the dose levels tested provide adequate margins of safety to accommodate any differences among the members of the category.

3.5 TEST PLAN TABLE

Chemical	Physical-Chemical Properties				
	Melting Point	Boiling Point	Vapor Pressure	Partition Coefficient	Water Solubility
CAS No. 100-52-7 Benzaldehyde	A	A	A, Calc	A, Calc	A, Calc
CAS No. 123-11-5 *p*-Methoxybenzaldehyde	A	A	A, Calc	A, Calc	A, Calc
CAS No. 121-33-5 *m*-Methoxy-*p*-hydroxybenzaldehyde	A	A	A, Calc	A, Calc	A, Calc
CAS No. 140-11-4 Benzyl acetate	A	A	A, Calc	A, Calc	A, Calc
CAS No. 120-51-4 Benzyl benzoate	A	A	A, Calc	A, Calc	Calc
CAS No. 93-58-3 Methyl benzoate	A	A	A, Calc	A, Calc	A, Calc
CAS No. 99-75-2 Methyl *p*-methylbenzoate	A	A	Calc	A, Calc	Calc
CAS No. 119-36-8 Methyl 2-hydroxybenzoate	A	A	A, Calc	A, Calc	A, Calc
CAS No. 2050-08-0 Pentyl 2-hydroxybenzoate	Calc	A	Calc	Calc	Calc
CAS No. 118-58-1 Benzyl 2-hydroxybenzoate	Calc	A	A, Calc	Calc	Calc

Chemical	Environmental Fate and Pathways			
	Photodegradation	Stability in Water	Biodegradation	Fugacity
CAS No. 100-52-7 Benzaldehyde	**Calc**	**NA**	**A, Calc**	**Calc**
CAS No. 123-11-5 *p*-Methoxybenzaldehyde	**Calc**	**NA**	**A, Calc**	**Calc**
CAS No. 121-33-5 *m*-Methoxy-*p*-hydroxybenzaldehyde	**Calc**	**NA**	**Calc**	**Calc**
CAS No. 140-11-4 Benzyl acetate	**Calc**	**Calc**	**A, Calc**	**Calc**
CAS No. 120-51-4 Benzyl benzoate	**Calc**	**Calc**	**A, Calc**	**Calc**
CAS No. 93-58-3 Methyl benzoate	**Calc**	**Calc**	**A, Calc**	**Calc**
CAS No. 99-75-2 Methyl *p*-methylbenzoate	**Calc**	**Calc**	**Calc**	**Calc**
CAS No. 119-36-8 Methyl 2-hydroxybenzoate	**Calc**	**Calc**	**A, Calc**	**Calc**
CAS No. 2050-08-0 Pentyl 2-hydroxybenzoate	**Calc**	**Calc**	**A, Calc**	**Calc**
CAS No. 118-58-1 Benzyl 2-hydroxybenzoate	**Calc**	**Calc**	**A, Calc**	**Calc**

Chemical	Ecotoxicity		
	Acute Toxicity to Fish	**Acute Toxicity to Aquatic Invertebrates**	**Acute Toxicity to Aquatic Plants**
CAS No. 100-52-7 Benzaldehyde	**A, Calc**	**A, Calc**	**A, T, Calc**
CAS No. 123-11-5 *p*-Methoxybenzaldehyde	**R, Calc**	**T, Calc**	**T, Calc**
CAS No. 121-33-5 *m*-Methoxy-*p*-hydroxybenzaldehyde	**A, Calc**	**T, Calc**	**A, R, Calc**
CAS No. 140-11-4 Benzyl acetate	**A, Calc**	**R, Calc**	**R, Calc**
CAS No. 120-51-4 Benzyl benzoate	**A, Calc**	**T, Calc**	**T, Calc**
CAS No. 93-58-3 Methyl benzoate	**A, Calc**	**A, Calc**	**R, Calc**
CAS No. 99-75-2 Methyl *p*-methylbenzoate	**R, Calc**	**R, Calc**	**R, Calc**
CAS No. 119-36-8 Methyl 2-hydroxybenzoate	**R, Calc**	**R, Calc**	**R, Calc**
CAS No. 2050-08-0 Pentyl 2-hydroxybenzoate	**A, Calc**	**A, Calc**	**T, Calc**
CAS No. 118-58-1 Benzyl 2-hydroxybenzoate	**A, Calc**	**R, Calc**	**R, Calc**

Chemical	Human Health Data					
	Acute Toxicity	Genetic Toxicity *In Vitro*	Genetic Toxicity *In Vivo*	Repeat Dose Toxicity	Repro-ductive Toxicity	Develop-mental Toxicity
CAS No. 100-52-7 Benzaldehyde	**A**	**A,**	**A**	**A**	**A**	**R**
CAS No. 123-11-5 *p*-Methoxybenzaldehyde	**A**	**A**	**R**	**A**	**R**	**R**
CAS No. 121-33-5 *m*-Methoxy-*p*-hydroxybenzaldehyde	**A**	**A**	**A**	**A**	**R**	**R**
CAS No. 140-11-4 Benzyl acetate	**A**	**A**	**A**	**A**	**A**	**A**
CAS No. 120-51-4 Benzyl benzoate	**A**	**A**	**R**	**A**	**R**	**A**
CAS No. 93-58-3 Methyl benzoate	**A**	**A**	**R**	**A**	**R**	**R**
CAS No. 99-75-2 Methyl *p*-methylbenzoate	**A**	**A**	**R**	**R**	**R**	**R**
CAS No. 119-36-8 Methyl 2-hydroxybenzoate	**A**	**A**	**A**	**A**	**A**	**R**
CAS No. 2050-08-0 Pentyl 2-hydroxybenzoate	**A**	**R**	**R**	**A**	**R**	**R**
CAS No. 118-58-1 Benzyl 2-hydroxybenzoate	**A**	**A**	**R**	**R**	**R**	**R**

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
T	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties
O	Other

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